

WASHINGTON STATE PATROL

CODIS LABORATORY STR ANALYSIS PROCEDURES

CRIME LABORATORY DIVISION

June 2016

Table of Contents

1.0	Introduction.....	4
2.0	CODIS Case Approach.....	7
3.0	Quality Assurance/Quality Control.....	12
4.0	Performance Check and Calibration of Instruments.....	15
5.0	Processing of Convicted Offender Samples.....	16
6.0	Wallac DBS Puncher Protocol – Electronic Barcode Record Tracking.....	17
7.0	DNA IQ™ Manual Extraction for Convicted Offender Samples.....	20
8.0	Qiagen BioRobot® Universal -- Introduction.....	21
9.0	Qiagen BioRobot® Universal -- Preparation of Samples for DNA IQ™ Extraction.....	22
10.0	Qiagen BioRobot® Universal – DNA Extraction Setup.....	23
11.0	Qiagen BioRobot® Universal – Quantifiler Setup.....	25
12.0	Qiagen BioRobot® Universal – Normalization of Quantified DNA.....	27
13.0	Qiagen BioRobot® Universal – Amplification Reaction Setup.....	28
14.0	Microcon® Concentration of DNA.....	29
15.0	ABI 7500 Sequence Detection System (SDS) Instrument Creating Detectors.....	30
16.0	ABI 7500 SDS Instrument – Plate Document Template SetUp.....	31
17.0	ABI 7500 Sequence Detection System (SDS) Instrument - Creating A Plate Document From A Template.....	32
18.0	Quantifiler® – DNA Quantification Reaction Preparation.....	33
19.0	Quantifiler™ and ABI 7500 SDS Instrument Data Analysis and Interpretation.....	34
20.0	Amplification OF STR LOCI – Identifiler® 25 µl.....	37
21.0	Amplification OF STR LOCI – Identifiler® 12.5 µL.....	38
22.0	Amplification Product Preparation for the 3500xL.....	39
23.0	Running Plates on the 3500xL Genetic Analyzer.....	40
24.0	GeneMapper® ID-X Setup.....	41
25.0	Guidelines for Manually Evaluating Convicted Offender DNA Typing Profiles.....	45
26.0	Guidelines for Evaluating Convicted Offender DNA Typing Profiles Using GeneMapper® ID-X V1.4 as an Expert System.....	53
27.0	CODIS Case File Content.....	55
28.0	Manual CODIS Case File Review With GeneMapper® ID-X V1.4.....	56
29.0	CODIS Case File Review With GeneMapper® ID-X V1.4 as an Expert System.....	58
30.0	TempGenius™ Wireless Data Acquisition & Monitoring System.....	60
31.0	Reagent Preparation.....	61
32.0	TempGenius™ Wireless Data Acquisition & Monitoring System Maintenance.....	64
33.0	UV Irradiator Operating Instructions.....	65
34.0	Wallac DBS Puncher; Instrument Maintenance.....	66
35.0	QIAGEN BioRobot Universal Maintenance.....	67
36.0	Biotek Plate Reader – Instrument Maintenance.....	68

37.0 ABI 7500 – Instrument Maintenance 69

38.0 9700 Thermal Cycler – Instrument Maintenance 70

39.0 3500xL Genetic Analyzer Maintenance 71

40.0 Maintenance Procedures – GeneMapper® ID-X V1.4 Server Version Only 73

41.0 Tuttnauer Tabletop Autoclave – Instrument Maintenance 74

42.0 Appendix A Administrative Procedures for Processing Offender Samples 76

43.0 References 81

44.0 Worksheets 83

45.0 Revisions 84

1.0 INTRODUCTION

Procedure development is an ongoing process. Consult the DNA Technical Leader for significant changes to any of the following procedures or for developing additional procedures. Proposed changes shall be reviewed by those affected by this manual prior to incorporation. Allowances (non-significant changes) may be made for adapting procedures (e.g. volume, tube size/type, spin times, incubation times) to accommodate unusual samples and situations. Allowances to adapt a standard procedure are a recognized part of lab work, as they may be necessary to meet the requirements of certain samples. These deviations must be scientifically sound and shall be documented in the case file.

The standard method of analysis of DNA conducted by the Washington State Patrol CODIS Crime Laboratory (WSPCCL) for the convicted offender database is by the analysis of Short Tandem Repeat (STR) regions by Polymerase Chain Reaction (PCR). The STRs examined by the WSPCCL include 15 loci, each having a four base pair repeat sequence. These are the 13 core loci selected by Combined DNA Index System (CODIS) and the National DNA Index System (NDIS), plus D2S1338 and D19S433. In addition, one non-STR locus, Amelogenin is included by the WSPCCL and NDIS. Amelogenin is a sex-linked locus with two alleles, one located on the X chromosome and one located on the Y chromosome.

PCR is a method used for the amplification of a specific DNA segment whereby two oligonucleotides (primers) anneal to opposite strands and flank a DNA region to be copied. The synthesis reaction is repeated for a number of cycles and results in the exponential accumulation of the specified DNA segment, the termini of which are defined by the 5' ends of the primers used in the reaction.

1.1 EXTRACTION

DNA suitable for PCR-based typing is currently obtained by the Promega DNA IQ™ extraction method.

1.2 HUMAN DNA QUANTITATION

The Applied Biosystems (AB) Quantifiler™ kits provide reagents and protocols necessary for the rapid and sensitive quantitation of human nuclear DNA. The role of the Quantifiler™ Human kit is to detect and quantify total human DNA. The genomic targets are PCR amplifiable sequence tagged sites on the human telomerase reverse transcriptase (hTERT) locus of chromosome 5. The procedure is quantitative PCR (qPCR) and is based on the real-time detection of a 5' fluorescent reporter dye that is cleaved and separated from a 3' non-fluorescent quencher incorporated on a DNA probe during PCR amplification. Also included in the kit is an internal PCR control (IPC) consisting of a non-naturally occurring DNA sequence that can detect the presence of PCR inhibitors. The qPCR is performed in the AB 7500 Real-Time PCR System instrument.

1.3 AMPLIFICATION

The polymerase used, Applied Biosystems AmpliTaq Gold®, requires heat activation. This has the advantage that the enzyme is not active during the setup of the samples. The formation of "primer dimers" is avoided and there is no time constraint placed upon the setup procedure. To activate the enzyme, there is an eleven (11) minute incubation at 95°C at the beginning of the amplification process.

Three steps are involved in each amplification cycle. First, the template DNA is made single-stranded by heat denaturation. In the second step, the temperature is lowered so that annealing of the oligonucleotide primers to the template may occur. This interaction is favored because, initially, the ratio of primer to template is large. Annealing of the PCR primers to the template DNA is responsible for the technique's high level of specificity. Under optimized conditions, annealing of complimentary DNA strands (primers) should only occur at those sites on the template flanking the region to be amplified. In this way, a sequence of several hundred bases can be selected from a background of, in the case of human DNA, 3×10^9 base pairs. In the third step, the temperature is raised to a point that favors both specific annealing of the primers and extension by the DNA polymerase. These three steps are repeated over and over again to

achieve a sufficient quantity of amplified product. The target DNA is amplified at an exponential rate if one assumes a 100% efficient PCR reaction. Even when carefully controlled, a PCR reaction is rarely, if ever, 100% efficient.

The AmpliTaq Gold[®] adds an extra nucleotide (usually adenosine) to the end of a strand of DNA. If this reaction only occurs partially, then DNA of two lengths, differing by one base pair, will be produced from the same template and primers, causing the appearance of a split peak. Having amplified product of uniform size is important in STR analysis, as it is the length of the final product that is detected. A length variance of one base pair is detectable by the system, and therefore undesirable. To avoid this, the addition can either be prevented or conditions chosen so virtually all the amplification product is converted. Since the latter is easier, a 60-minute soak at 60°C is included at the end of polymerization cycle to drive the addition reaction to completion.

A kit that contains all of the necessary components to amplify and detect Amelogenin and the 13 core STR loci used by CODIS is commercially available. It is possible to detect and distinguish the products of several loci amplified together. This is referred to as multi-component analysis. The detection of all 13 core STR loci plus Amelogenin, D2, and D19 is achieved by the use of the Applied Biosystems AmpF[®]STR[®] Identifiler[®] kit.

Identifiler[®] kit loci

Locus	Size Range (b.p.) ¹	Dye Color ²
D8S1179	128-168	Blue
D21S11	189-243	Blue
D7S820	258-294	Blue
CSF1PO	281-317	Blue
D3S1358	114-142	Green
TH01	169-189	Green
D13S317	206-234	Green
D16S539	234-274	Green
D2S1338	307-358	Green
D19S433	101-135	Yellow
vWA	157-197	Yellow
TPOX	218-242	Yellow
D18S51	273-341	Yellow
Amelogenin	107, 113	Red
D5S818	135-171	Red
FGA	219-267	Red

¹This is the size in actual base pairs; the estimated size generated by the CE unit will be different.

²The dyes used are 6' FAM (Blue), VIC (Green), NED (Yellow), PET (Red) and LIZ (Orange). For more information on the loci and dyes, please see the Identifiler[®] user's manual.

1.4 DETECTION

1.4.1 Multicomponent Analysis

Multicomponent analysis uses two separate properties to distinguish between loci: length variance and fluorescent labels.

Length Variance

The amplified product from each locus will vary in length, dependent upon the number of repeats found in a particular STR. By careful selection of primers, it is possible to produce amplified products for several loci, all of which fall into discrete size ranges. Thus a particular locus can be identified by the size of its alleles.

Fluorescent Labels

Different fluorescent labels can be attached to the primers used in the amplification process. The emission spectra of these labels are sufficiently different such that the contribution made by each label in a mixture can be calculated both quantitatively and qualitatively. Thus, even if two separate DNA fragments co-migrate, they can be differentiated by their fluorescent labels.

By careful selection of both primers and fluorescent dyes, several different co-amplified loci can be distinguished based both upon their fluorescence and the size of the fragment lengths. Applied Biosystems has constructed the Identifiler[®] kit, which contains five dyes. The green dye covers five discrete size ranges, the blue and yellow dyes cover four discrete size ranges each, and red covers three discrete ranges, one being a narrow range for the two alleles of Amelogenin. This allows for the detection of up to 16 loci at one time. The fifth dye, LIZ, is orange and is used to label the internal size standard.

1.4.2 Applied Biosystems Genetic Analyzers (CEs)

There are several instruments commercially available that will separate DNA fragments based upon their size and fluorescent labels. These instruments separate fragments using capillary electrophoresis. A window in the capillary is continually illuminated by a laser. As fragments of DNA with fluorescent labels pass by the window, they are excited and the resulting emitted light is collected and analyzed.

The WSPCCL currently uses AB 3500xL Genetic Analyzers.

1.4.3 Internal Size Standards

LIZ internal size standard is added to each sample prior to electrophoresis. The LIZ internal size standard consists of several amplified fragments of DNA, all of which have been sequenced and are of known length. The migration time of each fragment is recorded and this data is used to calculate an estimated length for the other DNA fragments that are detected.

1.5 INTERPRETATION OF RESULTS

The data generated is analyzed using the software program, GeneMapper[®] *ID-X*, available from Applied Biosystems.

This program converts the raw data from AB Genetic Analyzers into discrete peaks, assigns a size to those peaks based upon their estimated length in base pairs, and converts the estimated fragment sizes in base pairs to designated alleles by comparing the fragment sizes to the known alleles in a standard called an allelic ladder. The program also displays that data in the form of an electropherogram.

2.0 CODIS CASE APPROACH

Quality in the CODIS Laboratory is maintained using the Crime Laboratory Division (CLD) Operations Manual, the CLD Quality Manual, the DNA Quality Manual, the DNA Quality Assurance Standards, and following the protocols in this STR Analysis Procedures Manual. Resources for advice on case approach include senior Forensic Scientists, the CODIS technical lead, and the DNA Technical Leader.

2.1 ADMINISTRATIVE PROCESSING GUIDELINES

All convicted offender samples received in the laboratory are processed following the guidelines of the [Administrative Processing of Convicted Offender Samples](#) procedure. Convicted offender samples are collected by hundreds of collectors across Washington State using kits provided by the CODIS Laboratory. Kits are assembled in-house, and requests for these kits are handled by CODIS Laboratory staff. A detailed spreadsheet of kit requests is maintained by CODIS staff.

Completed kits are received through the mail or hand-delivery. Prior to acceptance by the lab, each completed kit is evaluated by a CODIS staff member. Information accompanying the DNA sample is assessed for completeness and accuracy. When a question arises about any of the submitted information, verification is done using the offender's criminal history record and/or contacting the collecting agency, prosecutor's office, or other law enforcement entity. Any information obtained using these sources is documented on the submission card along with the origin of the information and the date and initials of the individual recording the information. If a question arises about the quality of a sample, recollection can be requested. An electronic log is maintained of all rejected samples.

Each sample is assigned a unique laboratory number and is referenced only by that number thereafter. The electronic Convicted Offender Form is used for data entry into the LIMS system. By using the SID of the convicted offender when entering data, the entry program is able to verify whether or not a DNA sample has already been received for that individual and will auto-populate the electronic entry form with the individual's name and date of birth. For any sample that is determined to be a duplicate, the request generated upon entry of the sample is automatically cancelled. Cancelled samples are retained, but are not typically typed unless needed for a hit confirmation or as a quality control check. For any sample without a SID, every attempt is made to determine if one exists. If one does not exist, the sample is entered manually using an acceptable alternative identification number (see [the Administrative Processing of Convicted Offender Samples](#) procedure for more details). When a non-duplicate sample is entered with a SID, the criminal history record of that individual is updated to indicate a sample has been received.

A barcode for each sample is generated once the sample has been entered into LIMS. Barcode labels are applied to any individual component of the sample submission.

Once processed administratively, offender samples are stored in a secure location in the CODIS Laboratory until ready to be typed.

2.2 LABORATORY PROCESSING GUIDELINES

All laboratory work is conducted following the procedures in this manual and the CLD DNA Quality Manual. Any deviation from laboratory procedure is documented in the case file and approved by the DNA Technical Leader or designee. The CODIS technical lead (FS4) is designated by the DNA Technical Leader with the authority to approve acceptable minor deviations of STR interpretation guidelines and protocols. Before results may be released, peer review of a completed file is conducted in accordance with the CLD Quality Manual, this manual, and the DNA Quality Manual by a qualified DNA CODIS analyst.

The date on the top of extraction paperwork is the date the samples were prepared for extraction (either robotically or manually). For first-time extractions in manual sets or robotic extractions, this

date is also the date the set was started. The analyst will record the date testing was completed prior to technical review on the last page of the case file.

As a quality control measure, robot sets may contain random reanalysis samples. These are known duplicate submissions for existing samples that were previously typed. The goal random reanalysis sample amount is five percent of samples per completed samples in a year. Random reanalysis samples may also include felon hit confirmation samples.

GMID-X software is used to record information about samples that pass in a run folder but have flags or anomalies.

The samples are returned to the storage area once the analyst is done working with them.

2.2.1 Robotic Workflow

The non-sterile plates and pipette tips employed for robot procedures are UV irradiated prior to use.

Extraction, quantification set-up, normalization of the samples, and amplification set-up all occur on the robotic platform. Using the robots requires a minimum amount of analyst involvement. There may be occasion when some factor, such as a bubble in the line, affects the accuracy of small volume pipetting. If a quantitation result for a sample indicates a below than acceptable target concentration, but the sample yields a full profile, the low target does not need to be signed off by the DNA Technical Leader or CODIS technical lead.

Samples are punched into a 96-square well plate using an automated puncher. The barcode is read by the puncher computer (if the barcode is unreadable, the laboratory number is hand-entered into the computer) and associated with a particular well number. The sample maintains association with the well number through first analysis on a CE. A .pdf of the worklist tab of the Robot Templates will be generated prior to changing the Extraction Control or Reagent Blank values after copying the Puncher worklist into the Robot Templates.

After the samples have been punched, lysis buffer is added to the plate using a multi-channel pipette. The plate is then incubated in a water bath before being placed on a robot. It is acceptable to seal a plate after punching if an analyst wishes to wait to extract at a later date. The plate should be labeled with the set number, date, and the initials of the analyst.

During extraction, the analyst waits for the resin to be added by the robot and returns unused resin to the "used" resin container. Removing only the disposable trough from the robot deck ensures the robot will not pause during the extraction process due to the trough holder no longer being present on the robot or not being present in the correct position. Once the DNA in lysis buffer has been transferred from the square well plate to the flat bottom plate by the robot, the program pauses to allow the analyst to evaluate the flat bottom plate to ensure no punches were inadvertently transferred. If a punch is present, it is removed using clean forceps. The analyst returns the plate to the appropriate position on the robot and selects "ok" on the screen to indicate this check is complete and to re-commence the program. From this point on, the robot requires no other human interaction during the extraction process.

All other manual interactions with robot plates are handled at the robotic workstations.

The liquid in the wells of the elution plate that will contain the quantification standards and controls is removed; this ensures the quant standards and amplification controls will not be diluted unnecessarily.

During quantification setup, quantification standards are manually added to the quant plate in an appropriate hood. The positive amplification controls are also added in an appropriate hood during amplification setup. The negative amplification control is added at the robotic workstation using the TE buffer that was used for normalization.

For any sample that requires manual manipulation from a robot plate to another plate or tube, a witness is required only if no genetic information is present in the first pass data. The witness initials and dates the steps that were observed on the appropriate worksheet(s).

A CODIS analyst assigns the samples to themselves as a work list in LIMS prior to administrative review. A bar code for the work list is generated by LIMS and printing the first page of the work list. A mass-marking program allows the analyst to scan or enter their work list barcode, assign a set number to each of the samples, enter a result to Findings Entered, and indicate their technical and administrative reviewers. After the program is run, each of the samples in the work list will have this data associated with it and be marked "Admin Reviewed" in LIMS. At this point, the criminal history record, for those samples with SID numbers, is updated to indicate the DNA sample has been typed for each of these samples.

2.2.2 Manual Workflow

Rather than constructing a work list for a manual sample set, the analyst may individually assign the samples to themselves in LIMS. When analysis is complete, they add a result to the Findings section and mark the sample Draft Complete. Additionally, the set number the sample was typed in is indicated in the Notes field of the Case Info tab. The technical reviewer individually marks each sample "Tech Review" and the administrative reviewer individually marks each sample "Admin Review".

All tubes used in manual lab work are run in an autoclave and subsequently UV irradiated.

Each DNA sample is evaluated when determining how much to take for extraction. For blood samples, 3 Harris punches is common. For buccal samples, evaluation of the FTA paper for an appropriate color change is conducted. If there was a good color change on the paper, 5 Harris punches is common. If a sample exhibits little to no color change or the FTA paper does not appear disturbed, it is appropriate to take a cutting. For larger quantities of sample taken, a larger volume of lysis buffer is required. The reagent blank should contain the same volume of lysis buffer as the sample that had the most buffer.

If all of a sample is consumed, "sample consumed" along with the date and analyst's initials is written on the outside of the sample envelope.

The reagent blank and negative amplification control are amplified at the maximum volume allowed for the amplification. At minimum, the maximum volume taken for a DNA sample is used for the reagent blank.

When labeling tubes for amplification, the analyst will do so either behind a shield, with a mask, or in a hood to prevent contaminating the tubes with their own DNA.

2.2.3 Guidelines for Microcon[®]

In instances where multiple extractions have produced weak results, the extractions may be combined in a microcon to concentrate the DNA.

When the Quantifiler™ IPC is inhibited for a sample, or the sample appears degraded in the genotype results, the sample may also be microconned (even if only one extraction had been conducted up to that point).

When using a microcon, the associated reagent blanks for individual extractions are microconned at the same time. The date of the microconned reagent blank is the date the microcon was conducted.

2.2.4 Data Analysis

Ladders and controls are evaluated before conducting data analysis of convicted offender samples.

When anomalies are observed in the genotyping results of a sample they are verified/confirmed following the guidelines in the Guidelines for Evaluating Convicted Offender DNA Typing Profiles Using GeneMapper[®] ID-X protocol.

2.2.5 Alleles Below Reporting Threshold

Alleles below threshold may not be reported for entry into CODIS. Refer to the CODIS SOP for minimum number of allele requirements for convicted offender samples. A manual CODIS table is generated for samples that are not NDIS eligible or are missing D2 and/or D19 following the requirements listed in section [28.7 CODIS Database](#) of this manual.

If an incomplete profile is generated for a hit confirmation, the resulting alleles may be reported for exclusionary purposes even if there is fewer than the allowable minimum number of loci.

2.2.6 Microvariants and Tri-alleles

Careful evaluation of potential microvariants and tri-alleles is conducted by the CODIS analyst. In some instances, particularly with locus D2S1338, a microvariant allele for a locus extends into a neighboring locus. In instances where a locus is a homozygote and the neighboring locus is a tri-allele, peak heights are considered to verify if one of the alleles in the tri-allele might actually be a second allele for the homozygous locus. STRBase is also consulted for previously reported tri-alleles at a particular locus.

If an extreme peak imbalance (less than 45%) is noted for a heterozygous locus that is adjacent to a homozygous locus, care is taken to see if one of the alleles might be associated with the homozygous locus. When confirming the peak imbalance in this scenario, it may be necessary to amplify the sample in a full volume reaction.

A reinjection of a sample suspected of being a microvariant or tri-allele may be performed to verify the anomaly (see 30.1.2.iii, in this manual, for a detailed description of when re-injection is necessary). STRBase is consulted to determine if the microvariant or tri-allele had been previously reported. To serve as a confirmation, the microvariant or tri-allele should be reported using a CE platform and the Identifiler™ amplification kit. If the microvariant or tri-allele had not been previously reported under these conditions, a re-extraction is performed. If the same genotype results with re-extraction, the microvariant or tri-allele is reported in the file. After peer review, the not-previously-reported microvariant or tri-allele is submitted to STRBase.

2.2.7 Contamination

In instances of a suspected contamination event where the questioned PCR product is above analytical threshold, troubleshooting is done to determine where in the laboratory process it may have occurred. The CODIS contamination flowcharts in the DNA Quality Assurance Manual are consulted to assist with troubleshooting. Reanalysis of samples or controls containing suspected contamination below analytical threshold is not required. The procedure for evaluating contamination using the CODIS database is outlined in the CLD CODIS Manual. Contamination events above analytical threshold will be documented in the CODIS Contamination Log. Contamination should be reported to the CODIS technical lead as soon as possible.

2.2.8 Failed Samples

If a full profile is not generated for a sample after consuming the entire sample, the sample is considered to have failed. The request is cancelled in LIMS. A note about the sample failing or being SDIS-only is entered into the Notes field of the Case Info tab. If a duplicate is available that has not been typed, the analyst will un-cancel the request and type the duplicate sample at their next available opportunity. If the sample does not have a duplicate, the flags are changed to reflect a “no” result for the DNA Taken and Typed fields. The collecting agency or other law enforcement entity is contacted to see if the individual is still incarcerated to recollect the sample. Whether or not a sample can be recollected and the source of the information is documented on the Run Folder Review worksheet. When an agency agrees to recollect a sample, the information is recorded on an electronic log for recollection requests. It is the responsibility of the individual requesting sample recollection to follow up with an agency to ensure recollection occurred.

2.2.9 Composite Profiles

In instances where a sample has been consumed and a full profile was not developed, but a more complete or full profile may be constructed by considering multiple results of that sample, those iterations may be used to generate a composite profile. For a locus to be considered for use in the composite profile, it must meet reporting requirements as described in this manual.

A manual table is generated following the requirements listed in section [28.7 CODIS Database](#) of this manual. All run folders used for the composite profile will be indicated on the Run Folder Review sheet.

3.0 QUALITY ASSURANCE/QUALITY CONTROL

3.1 DNA ISOLATION AREAS

1. All solutions used in DNA isolation are prepared according to the protocols in the Reagent Preparation section of this manual.
2. All glassware to be used in preparation of critical reagents will be autoclaved. If a solution itself is to be autoclaved, the glassware used in its preparation need not be autoclaved beforehand. Pipette tips and microcentrifuge tubes that are sterilized (as in the manufacturing process) need not be autoclaved.
3. Gloves will be worn where appropriate.
4. Instruments used to cut or handle stains (scissors, forceps, scalpels, Harris punch) should be cleaned before each use.
5. Use filtered pipette tips when pipetting any solutions.
6. Care should be exercised when inserting pipettes into reagent containers. Stock reagents should be poured from the reagent bottle into a smaller container from which pipetting should be done. Avoid touching the container with the barrel of the pipette.
7. The work area should be cleaned after each use with 10% bleach or other appropriate cleaner.
8. The interiors of microcentrifuges should be cleaned periodically.

3.2 PCR SET-UP AREA

1. Gloves will be worn when working in the PCR set-up hood.
2. Set up the PCRs according to protocol. Use only the pipettes dedicated to the PCR set-up area. Use sterile, filtered pipette tips. Dispose of pipette tips in a waste container in the hood.
3. The hood will be cleaned on a regular basis with a 10% bleach solution (or other appropriate cleaner) and then rinsed with water (if using bleach). Pipette barrels will also be wiped regularly with a 10% bleach solution (or other appropriate cleaner). Additionally, the UV light in the hood (if so equipped) will be utilized after each use of the hood.

3.3 AMPLIFICATION AND PRODUCT ANALYSIS AREA

1. All solutions used in the amplification and product analysis area are prepared according to the reagent manual protocol.
2. Gloves will be worn where appropriate when working in the amplification room.
3. Only dedicated lab coats will be worn when working in this area.
4. Handling of PCR products will be done with dedicated pipettes and filtered pipette tips.
5. If bench top protective paper is used, it will be changed at least weekly.
6. Any PCR product or other items contaminated with PCR product will be discarded in the appropriate container in the PCR amplification/analysis work area.
7. PCR product may only be stored in a dedicated refrigerator or freezer.

3.4 CONTROLS AND STANDARDS

3.4.1 Reagent Blank

A reagent blank will be prepared each time a set of DNA samples is extracted. The reagent blank will be prepared like the other samples being extracted, except no source of DNA will be added to this sample. Any generated reagent blank shall be amplified using the same primers, instrument model, and concentration conditions as required by the sample(s) containing the least amount of DNA. The amplified reagent blank shall be typed using the same instrument model, injection conditions, and most sensitive volume conditions of the extraction set.

In accordance with FBI QAS Standard 9.5.3.2, if multiple reagent blanks are used within an extraction set, each reagent blank shall be quantified and at least one shall be amplified if any of the specimens associated with the extraction set will be amplified. If multiple reagent blanks are used and quantified with an extraction set, at minimum, the reagent blank that demonstrates the greatest signal, if any, shall be amplified and characterized.

When concentrating samples where a single extraction generated multiple reagent blanks, only one of those reagent blanks is required. The reagent blank taken forward should be one that, if formerly quantified, demonstrated the greatest signal, if any, unless that reagent blank was already demonstrated to be contaminated.

If the reagent blank associated with the extraction set or sample being amplified is depleted, an analyst shall not continue. The volume of sample amplified cannot exceed that of its reagent blank.

3.4.2 Positive Extraction Control

The positive extraction control is a sample from a known individual within the laboratory system who does not work in the CODIS Laboratory. Ideally, this control is run with every extraction through data analysis and should be treated as a regular sample. Every attempt should be made to get the positive extraction control to successfully genotype, but if it is the only sample delaying the completion of a set, testing may cease with an explanation provided in the case file.

Should a positive amplification control fail, a positive extraction control that is run in the same amplification may be used in its place to demonstrate the amplification worked as expected.

3.4.3 Positive and Negative Amplification Controls

At the amplification step, two extra tubes containing the amplification master mix will be prepared. To one tube the appropriate volume of extracted positive control DNA 9947A will be added, and to the other an equal volume of TE will be added.

A positive and negative amplification control will be included in every set of amplifications conducted.

3.4.4 Internal Size Standards

An appropriate internal size standard will be included with every sample prepared to be run on the CE. The data collected from this standard will be used to verify the quality of a particular injection and also be used to estimate the size of any DNA fragments present in the run. It is essential to have the data from this standard in order to process samples in the GeneMapper® ID-X program.

3.4.5 Allelic Ladder

An appropriate ladder will be included with every injection on the CE. A passing allelic ladder is essential in order to process samples in the GeneMapper® ID-X program.

3.5 QUALITY CONTROL OF REAGENTS

The following critical reagents shall be evaluated prior to use:

1. Promega DNA IQ™ kit
2. Dithiothreitol
3. TE
4. Quantifiler™ kit
5. Promega G147A for the QF standard curve
6. AmpF ℓ STR® Identifiler kit

3.6 REAGENT QUALITY CONTROL TESTING

Unless a quality control procedure indicates otherwise, a known DNA sample is used to perform the QC of a new lot number of reagent or kit before it may be used on convicted offender samples. The known DNA sample must be analyzed using the current laboratory protocols. The known DNA sample must type correctly and meet all data analysis parameters as required in the current laboratory protocols. If the sample does not type correctly or does not meet the analysis parameters, the reagent or kit may not be used for convicted offender analysis until the problem is resolved. Scientifically valid exceptions pertaining to meeting the analysis parameters can be made if approved by the CODIS technical lead in consultation with the DNA Technical Leader. The successful completion of the QC is documented on the reagent or kit and on the appropriate QC worksheet. All QC testing will be verified by another CODIS scientist to ensure that analysis parameters are met, the paperwork is filled out correctly, and the electronic data is present. This verification will be documented on the QC worksheet, which will then be saved as a PDF and stored with the electronic data.

4.0 PERFORMANCE CHECK AND CALIBRATION OF INSTRUMENTS

Routine maintenance procedures are described by instrument/equipment elsewhere in this manual (see Table of Contents). A maintenance item does not necessarily have to be performed on-schedule for an instrument that isn't being used at the time the maintenance is due. The operator will ensure all necessary maintenance items are performed prior to use. If annual maintenance is required of an instrument, it is not necessary to perform it within the year if the instrument is taken off-line.

The performance check and/or calibration of instruments demonstrate they are working properly and will produce the expected results. Performance checks will be conducted at least annually, and for critical instruments after calibration, maintenance, or repair. Specific performance check and/or calibration procedures may be found in the Instrument Maintenance log for that instrument (and in the Instrument Maintenance sections of this procedure manual). Written documentation of calibrations and performance checks will be maintained.

For each refrigerator/freezer/oven/water bath/heat block, variance should be established as per the [Equipment Calibration and Maintenance](#) section of the DNA Analysis Quality Assurance manual. If, when the temperature is checked, the equipment is functioning outside that range, use of the equipment in forensic testing should be stopped immediately and measures taken to return the equipment to function within its accepted range.

1. pH meters will be calibrated with known pH standards according to the manufacturers' instructions before they are used.
2. Balances will be checked at least annually by an external provider.
3. The operating temperature of refrigerators, freezers, and any other storage unit or area used to store reagents or samples that would degrade if not maintained at or below a given temperature will be recorded each working day or monitored using the TempGenius system.

The operating temperature of ovens, water baths, or other apparatus that is used to maintain a constant temperature of reagents or a reaction should be checked each working day, prior to its use, or monitored using the TempGenius system.

Semiannually, the thermometers/probes used to record these temperatures will be verified against a NIST traceable thermometer. The thermometer/probe can remain in service if it is within $\pm 4^\circ \text{C}$ of the NIST traceable thermometer. Alternatively, a thermometer can be used that is NIST traceable and certified for a specific time period as long as it is replaced or re-certified before that period has expired.

4. NIST traceable thermometers will be re-certified annually by an appropriately qualified external agency.
5. Thermal cyclers are a critical instrument and will be performance checked semi-annually using the appropriate temperature probe and the directions provided by the manufacturer of the thermal cycler.
6. Annually, the probes used to check the calibration of thermal cyclers will be re-certified by an appropriately qualified external agency.
7. Pipettes will have their calibration checked annually by a qualified external agency.
8. The AB genetic analyzers are critical instruments. They are subject to annual maintenance by a qualified technician.

An instrument verification shall be performed on a CE unit prior to use on convicted offender analysis for a CE unit that is moved or when a technician provides repair or routine maintenance. This verification will consist of successfully running an amplification positive or other known sample, an amplification negative, and a ladder

on the instrument and, if the sensitivity may have been affected, the HID install standard performance check. Verify the sample genotyped correctly and meets all data analysis parameters as required in the current laboratory protocols. Documentation of this verification will be maintained by the laboratory.

A new instrument requires a performance check which includes: 1) an instrument verification, 2) a sensitivity check, and 3) a 1 base pair precision check (± 0.5) with a minimum of data from 6 ladders run per capillary.

Data from a performance check of a new CE instrument will be compared to the other instruments in the laboratory. Requests for approval of new CE instruments for DNA analysis will include the performance test results, precision testing results, and demonstration that the sensitivity is in the CODIS Laboratory's established range.

A new spatial shall be run if a repair/maintenance involved removing the capillary array from the detector, if the capillary is changed, or if the instrument is moved.

9. The AB 7500 Real-Time PCR System is a critical instrument with a monthly calibration check and semi-annual calibration following the manufacturer's instructions. Additionally, the 7500 is subject to an annual maintenance by a qualified technician.
10. The QIAGEN BioRobot[®] Universal is a critical instrument. Monthly and semiannual performance check/calibration programs shall be run according to manufacturer instructions. Additionally, annual maintenance is conducted by a qualified technician.

5.0 PROCESSING OF CONVICTED OFFENDER SAMPLES

DNA samples received by the CODIS Laboratory are treated as reference materials.

Buccal samples on FTA paper (from a WSP provided kit) from convicted offenders are collected and submitted to the Crime Laboratory by law enforcement facilities. In rare instances, blood samples are drawn instead. It has been requested that these samples be submitted in EDTA vacutainer tubes, accompanied by a Washington State Patrol Crime Laboratory Division Convicted Offender DNA Data Sheet (WSP-CL-549).

On receipt, each whole blood sample is prepared as follows:

1. Ten to twelve 75 μ l stains are prepared on Schleicher & Schuell 903 paper or other clean filter paper. The stains are air-dried, sealed in plastic bags, and frozen at -20°C to -80°C , pending analysis.
2. Any remaining whole blood is destroyed by pouring into a disinfectant solution. The destruction of each blood sample is witnessed.

DNA typing of the convicted offender blood samples is done using a portion of one of the prepared 75 μ l bloodstains. Similarly, DNA typing of convicted offender buccal samples is done using a portion of the submitted FTA card.

6.0 WALLAC DBS PUNCHER PROTOCOL – ELECTRONIC BARCODE RECORD TRACKING

This protocol is for taking punches from buccal samples on FTA[®] paper and blood stains on S&S filter paper typically provided as samples from convicted offenders when an electronic record of the barcode/lab numbers is used as the tracking record.

6.1 STARTING THE WALLAC DBS PUNCHER SOFTWARE PROGRAM:

1. Turn on the computer.
2. Double-click the “Wallac DBS Puncher” icon to open the software program.
3. From the program menu, select “Tools > User Level”.
 - a. Select the User Level of “Advanced”.
 - b. Enter the password.
 - c. Click “OK” to close User Level window.

6.2 CREATING A NEW PUNCHING PROTOCOL:

A protocol serves as a template. It is ideal to have a few protocols pre-established rather than creating a new protocol for each set of samples.

1. Select Tools > Protocol Editor.
 - a. With the General tab selected, click New.
 - b. Fill in the following information:
 - i. Protocol name: A unique name to identify the protocol.
 - ii. Max number of plates: 1
 - iii. Punch head size: 4.7 mm (3/16”)
 - iv. Position of 1st plate: Right
 - v. Place a check-mark in Twin plate protocol field.
 - vi. Punches to each well: in the first window, enter the number of punches that will go into the sample plate (plate on the RIGHT). In the second window, enter the number of punches that will go into the waste plate (plate on the LEFT) - this value may be 1 even if more punches are selected for the sample plate.
 - vii. Patient replicates: 1
 - viii. Leave the Read plate barcodes field unchecked.
2. Select the Plate Maps tab:
 - a. Plate Number and Plate Map Type should be left as the defaults: 1 and Independent, respectively.
 - b. Highlight each well (or wells) and designate them as one of the following: Extraction Control, Empty, Reagent Blank, or Sample.
3. Select the Worklists tab and set up as follows:
 - a. Worklist file type: Simple
 - b. Worklist file path: C:\DATA
 - c. Worklist file name: This is the electronic name of the output file. Use the set name.
 - d. Printout type: None

- e. Leave the last four fields (for the waste plate) as the defaults: <none>, blank, blank, and <none>, respectively.
- f. Click OK

6.3 PUNCHING:

1. Clean the puncher head
 - a. Remove the trigger plate and wipe it with a soft cloth or Kimwipe[®] tissue moistened with detergent.
 - b. Remove the semi-circular cover plate, press the spring-loaded button, and pull out the puncher head. (Note: the puncher piston will fall out of the puncher head if turned upside down during handling.)
 - c. Clean the puncher head with a brush or a jet of high pressure air.
 - d. To replace the puncher head, push in the spring-loaded button and make sure the narrow part of the rubber ring on top of the head engages with the metal fork that lifts the piston. Push the head back until it is firmly in position and release the button.
2. Turn on the puncher by using the switch on the top-right back-side of the instrument. The instrument will perform an automatic check of its functions.
 - a. Once on, the puncher asks whether it will be used in a procedure controlled by external control software (Slave, TTY) or as a Stand Alone puncher (S/A).
 - b. Select Slave mode and the screen should read "Under external control".
3. Run a static ionizer unit over and under the trigger plate and over the 96-well plate and then place it near the punching area.
4. From the main screen, start the protocol by doing the following:
 - a. Select the desired protocol from the Select "Protocol" field.
 - b. Change the name of the worklist to the set number.
 - c. The Worklist mode should be designated as "Start new".
 - d. Click the "Start Punching" button.
 - e. Follow the prompts on the computer screen and load the sample plate on the RIGHT side of the DBS Puncher oriented so A1 is in the back right of the puncher and press OK on the puncher screen.
 - f. Load the waste plate on the LEFT side of the DBS Puncher oriented so A1 is in the back right and press "OK" on the puncher screen.
 - g. The DBS Puncher will calibrate and position itself over the first well to be punched.
5. Start punching:
 - a. Turn the barcode reader on by pushing the button on the top and scan the barcode of the first sample by presenting it to the barcode reader and waiting for the beep.

- b. Alternatively, if the barcode cannot be read, the number may be manually entered. Type the number including the dash and press enter
 - c. When the sample number has been successfully read, place the sample under the punch head.
 - d. Instruct the instrument to punch by doing one of the following:
 - i. Select "Punch" on the DBS Puncher screen or
 - ii. Gently press down on the trigger plate below the puncher head or
 - iii. Press the foot pedal
 - e. When one punch has been punched, the instrument will indicate that it is ready to punch an additional punch into the well from the sample (if the protocol indicates multiple punches) or will move to the waste plate for a cleaning punch.
 - f. Once the waste plate is under the punching area, use compressed air to clean the punch stand of any debris. Use clean filter paper for the cleaning punch between each sample.
 - g. The puncher will automatically move the plate to the next sample well and will prompt the user to read the next barcode.
 - h. To check the plate mid-program, click "Check Plate" to bring the plate to the loading position. The plate may be removed and visually checked to assure that everything is correct. Return the plate to the proper position and use the "Resume" softkey to continue punching.
6. When all samples have been punched as designated on the sample sheet:
 - a. Click the "Plate Completed" button on the computer screen.
 - b. Follow the prompts on the computer screen.
 - c. Click "OK" once the computer screen indicates "You have punched all the plates to this worklist."
 - d. The worklist is saved to the Data folder on the computer and is used as the sample tracking record.
7. When the punching session is over, turn the puncher off using the switch on the back of the instrument.

7.0 DNA IQ™ MANUAL EXTRACTION FOR CONVICTED OFFENDER SAMPLES

This method is for DNA extraction from buccal samples on FTA® paper and blood stains on filter paper typically provided as samples from convicted offenders. This method may also be used for swabs, foam-applicators, and gauze or other cloth materials. The DNA IQ™ extraction system (Promega) is a DNA extraction method that uses a paramagnetic resin to capture DNA. Provided there is sufficient DNA present to saturate the resin, elution into 100 µL will give a concentration of approximately 1 ng/µL. A range of elution volumes can be used to vary the concentration of the resulting DNA extract. Depending on the nature of the sample being extracted, the elution volume or the amount of sample extracted can be adjusted.

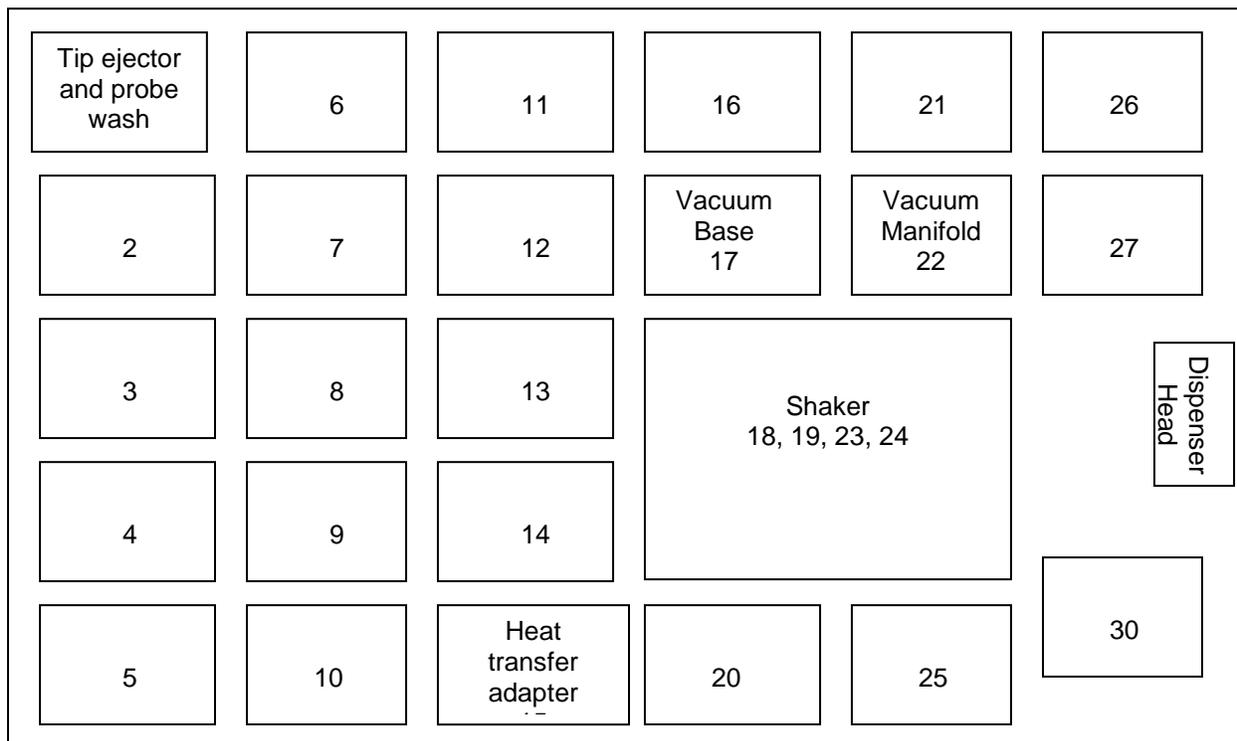
1. Place sample into a 2 mL tube basket assembly or, for smaller sample sizes, a 1.7mL microcentrifuge tube (or other tube/tube basket assembly that has been performance tested and found to be suitable).
2. Add 150 µL (or up to 300 µL for larger sample amounts) prepared Lysis Buffer +DTT and incubate at 95°C for 30 minutes.
3. Using a sterile technique, remove any punches from the tubes. If using a tube/basket assembly, transfer sample into the basket insert and place the insert back into the tube. Spin at maximum speed in a microcentrifuge for 2 minutes. Remove and discard the basket insert, including the basket contents.
4. Vortex stock resin bottle for 10 seconds. Add 3-7 µL re-suspended resin to sample. Keep stock resin re-suspended while dispensing to obtain uniform results. Vortex sample tube for 3 seconds and incubate at room temperature for 5 minutes.
5. Vortex sample tube for 2 seconds. Place sample tube on magnetic stand and carefully discard solution with a disposable pipette without disturbing resin.
6. Add 100 µL prepared Lysis Buffer +DTT and vortex tube for 2 seconds. Place tube in magnetic stand. Carefully remove and discard solution.
7. Add 100 µL prepared 1X Wash Buffer to tube and vortex for 2 seconds. Place tube in magnetic stand and carefully discard solution. Repeat for a total of three washes.
8. With lids open, air-dry 5-20 minutes at room temperature on the magnetic stand.
9. Add Elution Buffer (between 25 and 100 µL; usually 50 µL) and close lids. Vortex tube for 2 seconds and incubate at 65°C for 5 minutes.
10. Remove tubes from heat, immediately vortex for 2 seconds and place on magnetic stand. Remove DNA extract to a microcentrifuge tube for immediate use and/or storage. Samples may be stored in a refrigerator for a few days or in a freezer for long term storage.

8.0 QIAGEN BIOROBOT® UNIVERSAL -- INTRODUCTION

The QIAGEN BioRobot® Universal with QIAsoft™ v 5.0 software is used by the Washington State Patrol Crime Laboratory Division CODIS Laboratory to extract DNA, set up quantitation reactions, normalize the concentrations of the DNA, and set up the STR amplification reactions for convicted offender samples. The layout of the instrument work surface is designated numerically starting from the back of the instrument to the front in columns from left to right. There are six columns of five locations each. Location one in the back left is where the tips are ejected. Figure 1 illustrates this numbering system.

Figure 1: Numbering system on the Universal

Back of Robot



Front of Robot

The BioRobot Universal employs software (QIAsoft™) to manage the robot and allow development and execution of each protocol. There are five protocol environments that are accessible by users based on their user level. For more information on these environments, see the QIAsoft™ Operating System Users Manual.

The Maintenance Environment should be checked before each run to see if a maintenance procedure should be performed.

9.0 QIAGEN BIOROBOT[®] UNIVERSAL -- PREPARATION OF SAMPLES FOR DNA IQ[™] EXTRACTION

9.1 PREPARATION OF SAMPLES

1. Take punches from each sample, distributing them into a deep 96-well plate using the DBS Puncher. The size of the punch and number of punches taken are at the discretion of the scientist, but if using the 4.7 mm punch, no more than four punches should be taken per sample. Follow the [procedure](#) in this manual for operation of the DBS Puncher.
2. Add 150 μ L of Lysis Buffer + DTT to each well (including controls) of the deep well plate, seal the plate with sealing foil, and incubate for 30 minutes at 95°C. Continue with BioRobot[®] DNA IQ[™] Extraction Protocol.

10.0 QIAGEN BIOROBOT® UNIVERSAL – DNA EXTRACTION SETUP

1. Turn on the BioRobot® and start the QIAsoft™ Software.
 - a. Enter the username and password.
 - b. Open the “DNA IQ” protocol.
 - c. Click on the green arrow to start the program.
2. Start the program from the beginning and follow the instructions as given by the program. Default values are typical for this process:
 - a. Enter sample number – enter the sample number. Follow the instructions for placing racks on the robot.
 - b. Enter the volume of Lysis Buffer to be added in the second step.
 - c. Enter the volume of Wash Buffer to be added.
 - d. Enter the volume of Elution Buffer to be added – The acceptable range for the robot is 50 – 100 µL.
 - e. Place the elution rack in the appropriate location, remove the tape and leave the lid on.
 - f. Vortex the Resin and then fill the 20 mL trough with at least 5 mL. Be sure to vortex the resin well before manually pipetting into the trough. Use the resin in the resin bottle marked “reused”. If there is not enough, then add resin from a stock resin bottle of the same lot.
 - g. Put the 5 x 80 mL troughs in the trough holders.
 - h. The robot calculates the amount of reagents to put in each trough. It is acceptable to round up to the nearest 5 mL when putting the reagents in the troughs.
 - i. Fill the tip racks as described.
3. The robot will initialize and then begin extraction. During the extraction process:
 - a. Wait for the robot to finish dispensing the Resin and move the Reagent Tray to the heat plate.
 - b. Remove the trough, leaving the Reagent Tray in place and manually pipette remaining resin to the resin bottle marked “reused”.
 - c. The robot will pause after transferring the Lysis Buffer from the deep well plate to the flat bottom plate to allow the analyst to verify no sample punches were transferred into the flat bottom plate. Once the scientist has verified no punches were transferred, select OK on the computer screen to continue with extraction. If punches were transferred, remove them with clean forceps ensuring the forceps are cleaned between uses.
 - d. At this time it is acceptable to leave the robot running unattended; however it is recommended to periodically check on the run.
 - e. Once extraction is complete, discard the deep-well extraction plate and flat bottom plate and preserve any remaining buffers by covering the troughs with parafilm until the next use.
 - f. The samples can remain on the robot for quantitation setup.
 - g. If the samples will not be immediately used, put the lid on the plate and store in a refrigerator.

h. Store the plate in a freezer with strip caps for long-term storage.

11.0 QIAGEN BIOROBOT® UNIVERSAL – QUANTIFILER SETUP

Prior to setting up the ABI Quantifiler™ Human kit with the QIAGEN BioRobot® Universal, turn on the ABI 7500 Sequence Detection System (SDS) and associated computer.

1. Prepare the standard curve according to the instructions in this manual (Reagent Preparation section).
2. Prepare the Quantifiler™ PCR reaction mix in a tube using the following calculation:
Reaction mix: (# samples + standards) x 5.2 µL
Primer: (# samples + standards) x 4.4 µL
 - a. Be sure to vortex and spin the primer prior to dispensing and swirl the reaction mix prior to dispensing.
 - b. Vortex tube after combining reagents.
3. Turn on the BioRobot® Universal and associated computer and open the QIASoft™ Software.
 - c. Login to the software.
4. Open the appropriate quantification setup protocol. Follow the directions given by the robot:
 - a. Enter the sample number
 - b. Place the plates on the robot in their appropriate location and remove the lid from the elution tube rack.
 - c. When placing the optical plate on the robot, do not touch the tube portion or top of the optical plate, even with a gloved hand.
 - d. Enter the volume of Master Mix to be added per well – for a 10 µL quantitation, the default is 9 µL.
 - e. Place the tube with Master Mix on the cooling block, remove the lid.
 - f. Enter the volume of DNA to be added per well – for a 10 µL quantitation, the default is 1 µL.
 - g. Fill the tip racks as described.
5. Once the program is complete, set the optical plate into the optical plate holder. Be careful to not set the plate directly onto the lab bench.
6. Complete the Quantifiler™ set up as follows:
 - a. In a PCR prep hood, manually add the standards to the appropriate wells in the plate.
 - b. Cover the plate with optical adhesive film and use the plastic wedge to seal the plate; ensure no air bubbles are on the seal. Do not touch the top of the seal (even with a gloved hand).
 - c. If air bubbles are present, flick the plate to remove them (do not touch the tube or top part of the plate) or place plate and plate holder into the centrifuge and spin at 3000 - 3100 rpm for one minute.
 - d. Evaluate the plate to be sure there are no bubbles in the wells. If any remain, flick the plate and/or spin again until the bubbles are gone.
 - e. Push open the ABI 7500 SDS plate tray

- f. Place the 96 well plate in the ABI 7500 SDS instrument with well A1 in upper left corner.
 - g. Push the tray closed.
 - h. Follow the Quantifiler™ and ABI 7500 SDS Instrument Data Analysis and Interpretation SOP for collecting and analyzing data.
7. Turn off the ABI 7500 SDS instrument at the earliest convenience.

12.0 QIAGEN BIOROBOT[®] UNIVERSAL – NORMALIZATION OF QUANTIFIED DNA

1. Convert the Quantifiler[™] Human DNA results from the 7500 SDS instrument into an exportable file for the QIAsoft[™] operating system of the BioRobot Universal as follows:
 - a. Arrange the QF data by column with the following headings: Well, Sample Name, Qty, Final Con.
 - b. Convert all 0 values to 0.01.
 - c. Convert all exponents to decimal.
 - d. Round all concentrations to 2 decimal places.
 - e. Evaluate the data to determine the appropriate amount of DNA to target for amplification and enter this value into the Final Con column. For samples with reasonable results, a target of 0.2 ng/μL (acceptable range is 0.03 – 0.4 ng/μL) may be used in a 12.5 μL amplification and a target of 0.15 ng/μL (acceptable range is 0.03 – 0.8 ng/μL) may be used in a 25 μL amplification. Note: each sample can have varying concentrations. If varying concentrations are used for each sample rather than targeting a particular value for the entire plate, save the report generated by the robot electronically to include in the case file.
 - f. Save the file as a “.csv”.
 - g. Include the word “Norm” in the save file (e.g. 07-1 Norm.csv).
 - h. Transfer the file to the User Data folder on the BioRobot[®] computer.
2. From the QIAsoft[™] Software, open the “Normalizaton” protocol and follow the directions of the robot:
 - a. Enter the name of your file – Be sure to include the .csv extension when entering your file name (e.g. 07-1 Norm.csv).
 - b. Enter the final volume – this is the final volume for the normalized samples; enter at least 30 μL for optimal pipetting.
 - c. Enter sample number – this is the total number of samples being normalized. Count the standards as samples.
 - d. Place a 5 x 80 mL trough with TE on the trough holder.
 - e. Fill the tip racks appropriately.
 - f. Place your samples in the elution rack with the lid off and the PCR plate (normalization plate) in the appropriate location on the robot. Label the normalization plate appropriately.
 - g. The robot will run the calculations for normalization of your samples. If an error occurs, go back to the .csv file and be sure the values are appropriate.
3. Once the program is complete, cap and freeze the remaining extracts.
4. If not immediately using, seal the normalization plate and store in a refrigerator for short-term storage or freezer for long-term storage.

13.0 QIAGEN BIOROBOT® UNIVERSAL – AMPLIFICATION REACTION SETUP

1. Prepare the master mix for the Identifiler® amplification kit in a 1.7mL tube.

- a. Use the following calculation for 12.5 µL amplification reactions:

Reaction mix: 5.75 x (# of samples)

Primer: 2.9 x (# of samples)

Taq: 0.3 x (# of samples)

- b. Use the following for 25 µL amplification reactions:

Reaction mix: 11.5 x (# of samples)

Primer: 5.75 x (# of samples)

Taq: 0.6 x (# of samples)

Be sure to vortex and spin each component before use and vortex and spin the master mix after preparation.

2. On the BioRobot® computer select the appropriate amplification setup protocol. Follow the protocol instructions on the BioRobot® computer:
 - a. Select the Samples Below – a graphic of a 96 well plate is visible; highlight by column the number of samples.
 - b. The program describes the process.
 - c. Follow the instructions for the layout setup for the robot.
 - d. Enter the Master Mix volume – for a 12.5 µL amplification, enter 8. For a 25 µL amplification, enter 15.
 - e. Enter template DNA volume per reaction.
 - f. Place the normalized samples on the robot.
 - g. Place an additional PCR plate on the robot for the amplification. Label the amplification plate appropriately.
 - h. Place the 1.7mL tube of amplification master mix in the reagent slot being sure to uncap the tube.
 - i. Put the appropriate tips on the robot.
 - j. If adding TE, select an amount to produce the desired target.
 - k. During the run, add TE to the –C well from the trough TE was dispensed from during the normalization protocol.
3. When the run is complete, take the amplification plate to a PCR set-up hood to add the positive amplification controls and seal the PCR plate. Place the plate on the ABI GeneAmp® PCR System 9700 thermal cycler.
 - a. Select the appropriate program for a 13 or 25 µL amplification on the 9700 and start the amplification process.
4. Seal and freeze the remaining normalized samples.

14.0 MICROCON[®] CONCENTRATION OF DNA

1. Assemble a Microcon[®] Fast Flow concentrator unit. To the top of the concentrator, add 30-100 μ l TE, if desired. Transfer the DNA extracts to be concentrated to the top of the concentrator. The total volume of extract and TE added to the sample reservoir should be no more than 500 μ L.
2. Cap the concentrator and spin in a centrifuge at ~500 to 5000 x g for about 10-15 minutes or longer (settings can be adjusted according to centrifuge type and sample viscosity).
3. Carefully remove the concentrator unit from the assembly and discard the filtrate fluid from the filtrate vial. Return the concentrator to the top of the filtrate vial.
4. Add 200 μ l TE to the concentrator. Recap and spin the assembly in the centrifuge for ~10 minutes at ~500 to 5000 x g.

NOTE: Additional washes may be required to remove inhibitors that may be present and should be done on samples of extremely limited quantity.

If the microcon is being used to just concentrate or reduce sample volume, the addition of TE as a wash step is not necessary.

5. Remove the cap, if adding TE; add the desired final volume of TE to the concentrator.
6. Remove the concentrator from the filtrate vial and carefully invert the concentrator onto a new labeled retentate vial. Discard the filtrate vial.
7. Centrifuge the assembly at 1000 x g for ~5 minutes (or pulse briefly).
8. Discard the concentrator. Cap the retentate vial.

Samples are ready for human DNA quantitation. Use samples immediately for PCR or refrigerate samples for storage while the sample is actively being worked (avoid repeated freeze-thaw cycles). Freeze samples for long term storage. Prior to use for PCR, vortex and pulse spin samples.

15.0 ABI 7500 SEQUENCE DETECTION SYSTEM (SDS) INSTRUMENT CREATING DETECTORS

Before creating a plate document, detectors shall be created in the SDS software. Once the detectors are created, this step may be omitted in subsequent runs.

1. Launch the SDS software and open a new plate document.
2. Select Tools > Detector Manager.
3. In the lower left of the Detector Manager dialog box, select File > New to open the New Detector dialog box.
4. Create a detector for the Quantifiler™ Human kit:
 - a. Enter Quantifiler™ Human in the Name field.
 - b. Select FAM as the Reporter Dye.
 - c. Ensure (none) is selected as the Quencher Dye.
 - d. Click the Color square to select a color.
 - e. Click Create Another to add the Quantifiler™ Human detector and to reset the New Detector dialog box.
5. Create a detector for the IPC assay:
 - a. Enter IPC in the Name field.
 - b. Select VIC as the Reporter Dye.
 - c. Ensure (none) is selected as the Quencher Dye.
 - d. Click the Color square to select a color.
 - e. Click OK to add the IPC detector and to return to the Detector Manager box.

16.0 ABI 7500 SDS INSTRUMENT – PLATE DOCUMENT TEMPLATE SETUP

A plate document template greatly reduces the time required to set up a plate document. Typically, the quantification standards and detectors will already be in place in a previously agreed upon layout requiring only that the unknown samples and detectors be entered. Additionally, thermal cycler conditions and reaction volume settings may be attached to a template. Templates may also be created for situations that consistently require a plate document that differs from a day-to-day standard plate layout.

1. Launch the SDS software.
2. In the SDS software, select File > New to open the New Document dialog box.
3. The default settings in the dialog box should read Absolute Quantitation (Assay), 96-Well Clear (Container), and Blank Document (Template).
4. Apply the desired template settings to the plate document. This might include any of the following:
 - a. Apply Detectors to the plate document.
 - b. Apply Detectors for standards and for unknown samples.
 - c. Input names for the quantification standards.
 - d. Set thermal cycler conditions (delete the Stage 1, the 50°C pre-incubation).
5. Save the plate document as a template.
 - a. From the SDS software, select File > Save.
 - b. For Save as type, select SDS Templates (*.sdt).
 - c. Locate and select the Templates folder within the SDS software folder (C:\Program Files\SDS 1.2\Templates). Enter a template file name. This might be a generic name easily recognizable for subsequent runs or it might be a name specific to the plate set up.
 - d. Click Save.

NOTE: Don't omit wells on the template.

17.0 ABI 7500 SEQUENCE DETECTION SYSTEM (SDS) INSTRUMENT - CREATING A PLATE DOCUMENT FROM A TEMPLATE

1. Launch the SDS software.
2. In the SDS software, select File > New to open the New Document dialog box.
3. The settings in the dialog box should read Absolute Quantitation (Assay) and 96-Well Clear (Container). For Template, select an appropriate template from the list. If the template is not in the list, click Browse to locate the required template (i.e. this may also be an Excel template filled out in advance in Excel with the test sample info).
4. Complete the plate document setup. This may require any or all of the following depending on the information already contained in the template:
 - a. Add detectors to the plate document (see ABI 7500 Sequence Detection System (SDS) Instrument; [Creating Detectors SOP](#)).
 - b. Apply detectors for standards and for unknown using the well inspector from the view menu.
 - c. Enter sample names.
 - d. Set Thermal cycler conditions (if present, delete the Stage 1, the 50° C pre-incubation).
5. Before running the reaction plate, save the plate document as an SDS Document (*.sds) file.
 - a. From the SDS software, select File > Save.
 - b. Select the location for the file.
 - c. Enter a file name.
 - d. For Save as type, select SDS Documents (*.sds) then click Save.

NOTE: Don't omit a well before the data has been collected.

18.0 QUANTIFILER® – DNA QUANTIFICATION REACTION PREPARATION

The amount of PCR amplifiable human DNA is determined using the ABI Quantifiler™ Human DNA kit. This assay is performed using the ABI 7500 Sequence Detection System (SDS).

Gloves shall be worn when handling kits and kit components.

1. Calculate the volume of the Quantifiler™ PCR Reaction Mix needed to prepare the reaction master mix (5.3 µl per sample and standard). Calculate the volume of Quantifiler™ Human Primer Mix to prepare the reaction master mix (4.5 µl per sample and standard).
2. Thaw the primer mix completely, vortex 3 to 5 seconds and pulse spin briefly before opening the tube (thaw one tube from a kit at a time to minimize freeze/thaw cycles.)
3. Swirl the Quantifiler™ PCR reaction mix gently before using. Do not vortex.
4. Pipette the calculated volumes of components into an appropriately sized tube.
5. Vortex the master mix for 3 to 5 seconds, then pulse spin briefly.
6. Obtain a 96-well reaction plate and seat in the optical support base. The reaction plate shall not be rested on any surface without the base. The plate shall not be touched on the bottom of the wells.
7. Dispense 9.2 µl of the master mix into each reaction well of the reaction plate.
8. Add 0.8 µl of sample, standard, or control to the appropriate well of the reaction plate, as determined by the plate setup sheet.
9. Seal the reaction plate with the Optical Adhesive Cover. Care shall be taken not to touch the adhesive cover. The plastic applicator shall be used to seal the adhesive cover to the reaction plate. Drag the applicator across the adhesive cover several times to ensure a proper seal. Seal around the edges and remove the white strips on the adhesive cover.
10. Centrifuge the plate as necessary to eliminate bubbles.
11. Open the ABI 7500 SDS instrument and place the precision holder in the heat block.
12. Place the 96 well plate in the ABI 7500 with well A1 in the upper left corner.
13. Push the tray closed.
14. Follow the Quantifiler™ and ABI 7500 SDS Instrument Data Analysis and Interpretation SOP for collecting and analyzing data.
15. After run is completed, turn off the ABI 7500 SDS instrument at the earliest convenience.

19.0 QUANTIFILER™ AND ABI 7500 SDS INSTRUMENT DATA ANALYSIS AND INTERPRETATION

The ABI Quantifiler™ Human DNA Quantification Real-Time PCR assays using the ABI 7500 Sequence Detection System (SDS) is used to determine the amount of PCR amplifiable human DNA. The Internal PCR Control (IPC) in the assay monitors for the presence of PCR inhibitors and may indicate when it is appropriate to consider additional DNA sample clean up steps.

1. Launch the ABI SDS software.
2. Analyze a run after it is complete and reanalyze after you make any changes to the plate document (such as sample names).
3. Open the plate document to analyze.
 - a. Verify the analysis settings:
 - b. Select Analysis > Analysis Settings to open the Analysis Settings dialog box.
 - c. Verify the settings are as follows: Threshold 0.200000, Baseline Start (cycle) 6, and Baseline End (cycle) 15.
 - d. Select Analysis > Analyze or click the Analyze icon.
4. View the Standard Curve.
 - a. In the Results tab, select the Standard Curve tab.
 - b. In the Detector drop-down list, select the Quantifiler™ Human detector.
 - c. View the CT values for the quantification standard reactions and the calculated regression line, slope, y-intercept, and R2 values. The CT value is the cycle at which the amplification curve crosses the threshold. The lower the CT value, the more DNA detected.
 - d. The slope indicates the amplification efficiency; -3.32 is ideal (100% efficiency).

NOTE: The range of acceptable values is determined by the specific laboratory's validation for each instrument. Typical values are:

Typical Range: -2.9 to -3.3

Average Slope: -3.1

If the slope is outside the determined range then a check of the assay setup, software setup, reagents and instrument may be appropriate.

- e. R2 (correlation coefficient) indicates the statistical significance of the standard curve. An R2 value greater than 0.98 is desirable. If R2 is less than 0.98, check the following:
 - i. Quantity values entered for quantification standards in the Well Inspector during the plate document setup.
 - ii. Preparation of serial dilutions of quantification standards.
 - iii. Loading of reactions for quantification standards.
 - iv. Failure of reactions containing quantification standards.
- f. The standard curve slope should be within the typical range between runs (see 4.d. above) or parameters determined by performance testing on individual instruments can be used to establish the acceptable range and

the R2 equal to or greater than 0.98. Only one acceptable standard curve is required for analysis. If two duplicate standard curves are run, one of the duplicates in a standard sample can be removed from the standard curve if it is an outlier. This may be done for up to two of the standard samples as long as there is one sample from the duplicate set that is acceptable. If one of the two duplicate standard curve sample sets is not acceptable, then that set may be entirely removed and the remaining acceptable standard curve sample set may be used. These changes shall be documented in the case file.

NOTE: If the standard curve exceeds the observed ranges of slope as stated above (4.d.) and the R2 is less than 0.98, the quantification may not be optimal and consequently the standard curve may still be used, but only to provide limited assistance in making analytical decisions (i.e. approximating the dilution to use for the desired target amount of DNA to amplify).

5. View the Amplification Plot.
 - a. In the Results tab, select the Amplification Plot tab.
 - b. In the Detector drop-down list, select Quantifiler™ Human and IPC.
 - c. Select the appropriate samples in the table below the amplification plot.
 - d. Ensure the threshold value is set to 0.20, the default setting.
 - e. The amplification view displays cycle-to-cycle changes in fluorescence and is useful for determining if there is no DNA present or if inhibition is preventing the amplification of the DNA. If the IPC crosses the threshold within the range of the standard samples but the sample does not cross the threshold, then the amount of DNA in the sample is less than 23 pg/ul with no inhibition. If the IPC and sample do not cross the threshold, PCR inhibition is evident and sample clean up should be considered. If there is a weak Quantifiler™ amplification of the sample with no or a reduced amplification of the IPC (outside the range of the standard samples) partial PCR inhibition is suspected. Sample clean up and re-quantification may be appropriate (see Microcon® Concentration of DNA SOP). There may be cases where there is a very strong amount of DNA present and the IPC shows inhibition but a full DNA profile may still be obtained.

6. View the Report.
 - a. The report summarizes the quantity of DNA present in the samples.
 - b. In the analyzed plate document, select the Results tab then select the Report tab.
 - c. Select the reactions in the 96-well plate representation below the report to display the results in the report.
 - d. View the Qty column to determine the quantity of DNA in each sample. Samples with the same name will have their quantity values averaged.

7. To print or export the report.
 - a. In the Report tab of the results window, select Tools > Report Settings, then set up how the report is printed and exported.
 - b. Select File > Print to print the report.

- c. Select File > Export to export the report as tab-delimited text that may be opened later using spreadsheet software.
- 8. A negative sample result may have an amplification plot similar to a very low level DNA sample due to background. Compare plot to a negative well to confirm whether or not it is background.

Data for each case file shall be saved as an *.sds file. The sample info may be omitted and detectors removed for samples from other case files run on the same plate.

20.0 AMPLIFICATION OF STR LOCI – IDENTIFILER® 25 µL

- The following parameters shall be used to program the thermal cycler:

Activation	95° C	11 min
Number of cycles:	1	
Denaturation	94° C	1 min
Annealing	59° C	1 min
Extension	72° C	1 min
Number of cycles:	28	
Final extension	60° C	60 min
Hold	10° C	∞

NOTE: An additional program may be built into the thermal cycler of 60°C for 45 minutes. This may be used to re-extend a sample which exhibits a shoulder. If this program is used, it shall be documented in the case file and shall also be applied to the reagent blank control.

- Prepare the DNA samples to be amplified. Approximately 0.125 to 4.0 ng of template DNA may be targeted. Using the DNA quantification results, calculate the volume of sample needed to provide the desired amount of DNA to amplify.
- Prepare the components from the AmpflSTR® Identifiler® PCR amplification kit by vortexing and pulse spinning the reaction mix, AmpliTaq Gold®, control DNA, and primer set.
- Prepare the samples for amplification (the following step shall be performed in the dedicated biological PCR amplification hood).
 - Prepare a master mix. For each sample, add:
 - 10.5 µL reaction mix
 - 5.5 µL primer set
 - 0.5 µL AmpliTaq Gold®
 - Vortex the master mix and aliquot 15 µl per PCR reaction tube.
 - Add 10 µl of TE/DNA mixture (separately or together), control DNA, and/or TE buffer to the appropriate tubes.
- Load the tubes into the AB GeneAmp® PCR System 9700 thermal cycler and start the amplification process.

21.0 AMPLIFICATION OF STR LOCI – IDENTIFILER® 12.5 µL

- The following parameters shall be used to program the thermal cycler:

Activation	95° C	11 min
Number of cycles:	1	
Denaturation	94° C	1 min
Annealing	59° C	1 min
Extension	72° C	1 min
Number of cycles:	28	
Final extension	60° C	60 min
Hold	10° C	∞

NOTE: An additional program may be built into the thermal cycler of 60°C for 45 minutes. This may be used to re-extend a sample which exhibits a shoulder. If this program is used, it shall be documented in the case file and shall also be applied to the reagent blank control.

- Prepare the DNA samples to be amplified. Approximately 0.063 to 4.0 ng of template DNA may be targeted. Using the DNA quantification results, calculate the volume of sample needed to provide the desired amount of DNA to amplify.
- Prepare the components from the AmpflSTR® Identifiler® PCR amplification kit by vortexing and pulse spinning the reaction mix, AmpliTaq Gold®, control DNA, and primer set.
- Prepare the samples for amplification (the following step shall be performed in the dedicated biological PCR amplification hood).
 - Prepare a master mix. For each sample, add:
 - 5.25 µL reaction mix
 - 2.75 µL primer set
 - 0.25 µL AmpliTaq Gold®
 - Vortex the master mix and aliquot 7.5 µl per PCR reaction tube.
 - Add 5.0 µl of TE/DNA mixture (separately or together), control DNA, and/or TE buffer to the appropriate tubes.
- Load the tubes into the AB GeneAmp® PCR System 9700 thermal cycler and start the amplification process.

22.0 AMPLIFICATION PRODUCT PREPARATION FOR THE 3500XL

1. Prepare a mixture of Hi-Di™ formamide and GeneScan™ 600 LIZ® Size Standard v2.0 in a microcentrifuge tube using the following formulas:
 - a. (# samples +1) x 8.5 µL formamide
 - b. (# sample + 1) x 0.5 µL LIZ 600
 - c. Example: 58.8 µL LIZ 600 per 1 mL formamide

NOTE: Formamide is a teratogen. Be sure to wear gloves.

2. Vortex and spin briefly. Label the tube with the LIZ lot number, the preparer's initials, and the date.
3. Dispense 9.0 µL of LIZ+formamide mixture into each well of a MicroAmp® reaction plate that will be used. Wells that are injected, but contain no PCR product must be filled with either LIZ+formamide or formamide.
4. Add 0.1 – 2.0 µL of PCR product or allelic ladder to each well. PCR product may be diluted with TE buffer.
5. Cover the plate with a septum and spin briefly to remove any bubbles.
6. Denature samples on a 95°C heat block or thermal cycler for 3 minutes.
7. Snap-cool samples for 3 minutes.
8. Place the plate in a plate base and cover with a plate retainer.

23.0 RUNNING PLATES ON THE 3500XL GENETIC ANALYZER

1. On the Dashboard of the 3500 Series Software, click the 'Start Pre-Heat' button to turn on the oven.
2. Complete any maintenance that is required.
3. With the instrument door closed, push the tray button to bring the autosampler forward. Place any plates to be run on the autosampler. When the door is shut, the autosampler should return to its home position.
4. Navigate to the Main Workflow portion of the 3500 Series Software to create a new plate or import a plate from a template. The following parameters must be applied to each plate:
 - a. Assay: 5sec, 7sec, 10sec, 15sec, or 24 sec
 - b. Results Group: CODIS
 - c. File Name Convention: CODIS
5. On the 'Load Plate for Run' screen, verify that any plates on the instrument are linked correctly and start the run.
6. Wait until the 'Monitor Run' screen is displayed before leaving the instrument. Error messages (ex. expired reagent installed, not enough POP-4 remains for all injections, etc.) may appear before this screen is displayed; the run will not proceed until the messages are acknowledged.

24.0 GENEMAPPER® *ID-X* SETUP

The following procedure applies to *ID-X* users with Admin profile access and shall be applied to any Server copy of *ID-X*.

If an individual user name has not been created, log in as user “gmidx”. Otherwise, log in using the individual user name. Once an Admin profile user has been created, any changes to the software shall be done from that profile to maintain a more accurate audit trail record.

24.1 SETTING UP A USER ACCOUNT

1. Open Security Manager
2. Select the appropriate user account type:
 - a. For an administrator, select Databasing Tech Lead
 - b. For an analyst, select Databasing Analyst
3. Select Edit > Duplicate
4. In the name field, change the name to the CODIS login of the individual the account applies to.
5. Enter the full name of the individual under Full Name in the User Details section.
6. Select Pre-Expire for the password to prompt the new user to create a password upon their first login.
7. Select Set Password and create a generic password for the new user.
8. Select File > Save

For custom user groups, user accounts, or security groups, follow the steps as outlined in the GeneMapper *ID-X* Administrator’s Guide. Additional information about editing users can also be found in the Administrator’s Guide. Only an Admin level user with the input of the CODIS technical lead and/or the DNA Technical Leader may make these changes.

After any changes are made in the Security Manager, a copy of the settings should be exported as a backup. If more than one Server exists, the settings shall be imported to that computer to ensure they are the same (see the GeneMapper *ID-X* Administrator’s Guide for instructions on exporting and importing security settings).

24.2 SETTING UP THE AUDIT TRAIL CONFIGURATION

1. Open the *ID-X* software
2. Choose Admin > Audit Manager > Setting
3. Log in to Audit Manager
4. Choose “Allele” from the left navigation pane
 - a. Set “deleted” and “created” to “on”
 - b. Set “modified” to “silent”
5. Choose “Sample” from the left navigation pane
 - a. Set “deleted”, “OverrideSQ”, “modified, and “created” to “off”
 - b. Set “OverrideGQ” and “OverrideCGQ” to “silent”
 - c. Set “ALLELE” to “on”

24.3 SETTING UP THE ANALYSIS METHOD FOR CONVICTED OFFENDER ANALYSIS

1. Open GeneMapper *ID-X* Manager.
2. Select the Analysis Method tab and click New.
3. Select the General tab and enter the following:

CODIS Lab STR Procedures	Page 41 of 87	Revision June 1, 2016
Approved by CLD Commander	All Printed Copies Uncontrolled	Revision 22

- a. Name: ID_3500xL_<implementation date>
 - b. Security Group: Databasing Security Group
 - c. Instrument: 3500xL
4. Select the Allele tab and enter the following:
 - a. Bin Set: AmpFLSTR_Bins_v3X
 - b. Use marker-specific stutter ratio and distance if available: check box
 - c. In the Tetra column, change the following:
 - i. MinusA Ratio: 0.1
 - ii. MinusA Distance: From 0.5 to 1.5
 - d. Amelogenin Cutoff: 0.0
 5. Select the Peak Detector tab and enter the following:
 - a. Ranges:
 - iii. Analysis: Partial Range, 2100 to 10000
 - iv. Sizing: Partial Sizes, 80 to 400
 - b. Smoothing and Baseline: Light Smoothing, 51 pts Baseline Winder
 - c. Size Calling Method: Local Southern Method
 - d. Peak Amplitude Threshold: 150 for all dye colors
 - e. Min. Peak Half Width: 2 pts
 - f. Polynomial Degree: 3
 - g. Peak Window Size: 15 pts
 - h. Slope Threshold: start and end at 0.0
 - i. Normalization: uncheck the box
 6. Select the Peak Quality tab and enter the following:
 - a. Homozygous min peak height: 550.0
 - b. Heterozygous min peak height: 150.0
 - c. Max Peak Height (MPH): 40000.0
 - d. Min peak height ratio: 0.45
 - e. Max peak width: 1.5
 - f. Max expected alleles: 2 for autosomal/AMEL, 1 for Y markers
 - g. Allelic Ladder Spike: Enable detection, Cut-off Value 0.2
 - h. Sample Spike Detection: Disable
 7. Do not alter the default settings in the SQ & GQ Settings tab.
 8. Save the analysis method.

24.4 CHANGING THE MARKER SPECIFIC STUTTER RATIOS

1. Open Panel Manager.
2. Choose AmpFLSTR_Panels_v3X and Identifiler_v1.2X_3500xL
3. Enter the stutter percentages as outlined in the table below:

Identifiler Stutter Thresholds

Locus	Minus Stutter	Plus Stutter
D8S1179	8.8%	1.4%
D21S11	9.4%	2.4%
D7S820	8.2%	3.1%
CSF1PO	9.6%	3.1%
D3S1358	12.0%	2.4%
TH01	5.1%	0.0%
D13S317	10.0%	2.6%
D16S539	10.4%	2.6%
D2S1338	12.6%	2.9%
D19S433	14.4%	5.0%
vWA	12.6%	5.2%
TPOX	5.4%	0.0%
D18S51	17.0%	5.2%
D5S818	10.0%	1.6%
FGA	14.7%	4.3%

4. Click Apply and close Panel Manager.

24.5 SETTING UP PROJECT OPTIONS

1. From the Project Window, go to File > Project Options.
2. Select the General tab and choose the following:
 - a. Under Project, check Open Blank Project
 - b. Under Data Access Control, select Databasing Security Group
3. Select the Add Samples tab and choose the following:
 - a. Set Analysis Method to: Current Analysis Method
 - b. Set Size Standard to: GS600_LIZ+Normalization_(80-400)
 - c. Set Panel to: Identifiler_v1.2X_3500xL
4. Select the Analysis tab and choose the following:
 - a. Under Analysis Summary check:
 - i. Stop analysis and display Analysis Requirements Summary
 - ii. Stop analysis and display Allelic Ladder Analysis Summary
 - iii. View Analysis Summary
 - b. Under Quality Metrics Display, check Symbols
5. Click OK to save the Project Options.

24.6 IMPORTING LAB REFERENCE AND CUSTOM CONTROL PROFILES

1. From the Project Window, open the run folder containing the desired profile
2. Go to the Samples tab and ensure the Sample Name is the desired custom name for the reference or control
3. Select the sample and go to Tools > Add Profile > Lab Reference (in the case of a reference) or Custom Control (in the case of a control)

4. Click Close in the Add Profile Results dialogue box to save the profile

24.7 ESTABLISH SOURCE AND DESTINATION LABS FOR CODIS FILES

1. Go to Tools > CODIS Export Manager
2. Under Source Lab IDs, enter the source lab and select add
3. Under Destination Lab IDs, enter the destination lab and select add
4. Click OK

25.0 GUIDELINES FOR MANUALLY EVALUATING CONVICTED OFFENDER DNA TYPING PROFILES

The following guidelines are provided for evaluating convicted offender DNA typing profiles obtained by STR analysis using the AB AmpF ℓ STR[®] Identifiler[®] Amplification Kit, an AB Genetic Analyzer, and AB GeneMapper[®] ID-X software.

25.1 GENERAL GUIDELINES FOR EVALUATING PROFILES

1. Raw Data

- a. Typically the primer peak should rise and fall abruptly and the baseline should be flat and smooth. In problem samples, if the primer peak trails excessively or the baseline is significantly elevated, the sample should be re-injected.

2. True alleles

- a. A true allele is defined as a distinctive, sharp peak that is expected to be greater than the analytical threshold (detection threshold) in height, clearly visible above baseline noise, and typically having a size estimated between 100 and 400 bp. Background signals, insufficient resolution, and the possibility of allelic drop-out should be considered and could make the sample data inconclusive for data below the analytical threshold.
- b. Homozygous alleles are expected to appear as single peaks at least as high as the stochastic threshold (550 rfu).
- c. Heterozygous alleles are expected to appear as two peaks at least as high as the analytical threshold (150 rfu) and usually have a peak height ratio greater than 45 percent.
 - i. When interpreting alleles just above the analytical threshold, allelic drop-out should be considered.
 - ii. Should a peak height imbalance be less than 45%, the sample shall be re-injected for confirmation. The Amelogenin locus is an exception to this re-injection requirement. If a peak height imbalance less than 45% persists, the analyst, in conjunction with the reviewer, can determine if the imbalance is acceptable. Otherwise, the sample shall be re-amplified.
- d. If an off-ladder allele is observed, the allele may be accepted without additional analysis if the size quality of the sample is passing and the variant allele has been previously observed. Otherwise, any off-ladder allele will be confirmed by a second injection to ensure the sample ran correctly.
 - i. If the variant allele has not been previously reported, re-extract the sample to confirm the result. If a re-extraction is being performed, a second injection is not required for confirmation of the variant allele. The NIST STRBase site <http://www.cstl.nist.gov/div831/strbase> can be checked for non-published variant allele reports to see if the allele has been encountered previously.
 - ii. The following table should be used as a guide for outside the ladder allele designations.

D8S1179	<8	>18
D21S11	<24.2	>38
D7S820	<6	>14
CSF1PO	<6	>15
D3S1358	<12	>19
TH01	<5	>10
D13S317	<8	>15
D16S539	<5	>15
D2S1338	<15	>28
D19S433	<9	>17.2
vWA	<11	>21
TPOX	<6	>13
D18S51	<9	>26
D5S818	<7	>16
FGA	<18	>30

- iii. For off-ladder alleles above or below the limits listed in the table that are given an “OL” designation by *ID-X*, the applicable > or < will be used as the allele designation.
- iv. For manual CODIS tables, the lower and upper values as outlined in the table above must be used.
- e. If a tri-allele is observed and has been previously reported at the NIST STRBase site, it will be confirmed by a second injection. If the tri-allele has not been reported, the sample will be re-extracted for confirmation. A second injection is not required in this case.
- f. Abnormal sex chromosome variants (e.g. XXY) do not need to be re-tested.
- g. If a one base pair difference between two alleles cannot be resolved using typical methods like re-injection or re-amplification, the Polynomial Degree and Peak Window Size in the *ID-X* analysis method may be adjusted. This will be documented in the case file and the modified analysis method should be included in the electronic data.

25.2 ANTICIPATED PCR ARTIFACTS

If an artifact can be confidently characterized as one of the following anomalies, the sample does not need to be re-injected. If there is uncertainty about an anomalous peak, the sample should be re-run to ensure quality and accuracy of allele calls. Those anomalous peaks that are labeled by GeneMapper® ID-X as either an allele or artifact must be given the correct label so as to not be mistakenly entered as a true allele in CODIS. The appropriate designation for each artifact is listed under that artifact’s description below.

1. Spikes

- a. Spikes have a general appearance of being needle-like and are randomly seen in samples. Spikes typically disappear with re-injection. Commonly, spikes are observed in most or all of the dye colors, and generally at the same base pair location. Occasionally, single color spikes may also be observed. The heights of spikes (in rfu) usually vary between dye colors.
- b. If a spike is given a designation by GeneMapper® ID-X, the analyst must ensure it is an artifact designation and that it is labeled: spike.
- c. It is permissible for an analyst to override the Genotype Quality of a marker for spikes.

2. Pull-Up

- a. Pull-up artifacts are peaks that show up at the same base pair size or within a base pair equivalent on either side of the true allele, but in another color. Pull-up usually occurs under peaks with excessive signal or under strong alleles.
- b. For instances where pull-up is given an inappropriate designation by the software, the analyst must ensure the artifact label indicates: pull-up.
- c. It is permissible for an analyst to override the Genotype Quality of a marker for pull-up.

3. N-4 Stutter Peaks

- a. These peaks are normally one repeat smaller than the true allele peak. Threshold values (% of true allele) for considering peaks as n-4 stutter are:

Identifiler n-4 Stutter Thresholds

Locus	Minus Stutter
D8S1179	8.8%
D21S11	9.4%
D7S820	8.2%
CSF1PO	9.6%
D3S1358	12.0%
TH01	5.1%
D13S317	10.0%
D16S539	10.4%
D2S1338	12.6%
D19S433	14.4%
vWA	12.6%
TPOX	5.4%
D18S51	17.0%
D5S818	10.0%
FGA	14.7%

- b. The analyst, in combination with the reviewer, can determine if a minimal deviation in stutter percentage is acceptable. Should stutter exceed the threshold greater than a minimum deviation, it will be evaluated jointly with the CODIS technical lead or DNA Technical Leader to determine if the potential of a weak heterozygote or tri-allele exists. Otherwise, the sample will be re-amplified. If the peak persists and is not noted on STRBase, the sample will be re-extracted.
- c. For acceptable elevated stutter peaks that are given a designation by GeneMapper® ID-X, use the artifact designation elevated stutter.
- d. It is appropriate for an analyst to override the Genotype Quality for a marker exhibiting elevated stutter if the conditions in 3.b are met.

4. N+4 Stutter Peaks

- a. These peaks are usually one repeat larger than the parent peak. Threshold values (% of true allele) for considering peaks as n+4 stutter are:

Locus	Validated Plus Stutter Percentage ^o
D8S1179	1.4%
D21S11	2.4%
D7S820	3.1%
CSF1PO	3.1%
D3S1358	2.4%
TH01	0.0%
D13S317	2.6%
D16S539	2.6%
D2S1338	2.9%
D19S433	5.0%
vWA	5.2%
TPOX	0.0%
D18S51	5.2%
D5S818	1.6%
FGA	4.3%

^o Taken from the WSPCCL n+4 study.

- b. N+4 can be observed at percentages higher than this amount and should be interpreted with caution. When in doubt, an analyst shall re-amplify the sample in a 25 µL amplification reaction for confirmation. If uncertainty still exists, the CODIS technical lead or DNA Technical Leader must be consulted.
- c. For acceptable elevated n+4 peaks that are given a designation by GeneMapper[®] ID-X, use the artifact designation elevated stutter.
- d. It is appropriate for an analyst to override the Genotype Quality for a marker with an n+4 peak provided the conditions of 4b are met.

5. Shoulders

- a. Shoulders flank the sides (one or both) of a true allele.
- b. If the shoulders are given a designation in GeneMapper[®] ID-X, the sample shall be extended, re-run at a shorter injection time, or re-amplified using less template DNA. This requirement does not apply to the Amelogenin locus or to shoulders 10% or less of the allele.
- c. It is not appropriate for an analyst to override the Genotype Quality for a marker with shoulders (with the exception of Amelogenin and shoulders 10% or less of the allele).

6. Formamide/Dye Blobs

- a. Raised baseline areas may represent formamide/dye blobs which usually occur at the same base pair location in multiple samples of the run.
- b. An analyst must ensure a dye blob given an artifact or allele designation by GeneMapper[®] ID-X has an artifact designation of: dye blob.
- c. It is appropriate for an analyst to override the Genotype Quality for a marker with a dye blob.

NOTE: When there is any uncertainty about an anomalous peak, the sample must be re-run to ensure quality and accuracy of the allele calls.

25.3 QUALITY FLAGS

Quality flags will be triggered if a sample does not meet the pre-set requirements or user-defined requirements for the software. Below is a list of potential flags and the conditions under which they could be overridden by an analyst.

1. Sample-level Quality Assessment
 - a. SOS – Sample Off-scale
 - i. May be overridden if any anomalies observed do not affect the quality of the genotyping result.
 - b. SQ – Sizing Quality
 - i. The sample may be accepted if the genotype of the sample and quality of the data is unaffected, but the flag should not be overridden until the technical reviewer also evaluates the sample.
 - c. MIX – Mixed Source
 - i. May be overridden if the sample is confirmed as a tri-allele or chimera.
 - ii. May be overridden if the analyst determines the flag was thrown due to the software designating artifacts as alleles
 - d. OMR – Outside Marker Range
 - i. May be overridden once the microvariant is confirmed. It is important that the analyst assigns the allele to the appropriate locus.
 - ii. May be overridden if the peak is determined to be an artifact by the analyst
 - e. SSPK – Sample Spike
 - i. May be overridden in samples and controls.
 - ii. May not be overridden in ladders.
 - f. CGQ – Composite Genotype Quality
 - i. May only be overridden by a peer reviewer as confirmation of agreeing with the analyst's changes to the sample.
 - ii. May not be overridden for ladders.
2. Marker-level Quality Assessment
 - a. OS – Off-Scale
 - i. May be overridden if any anomalies observed do not affect the quality of the genotyping result.
 - b. BIN – Out of Bin Allele
 - i. May be overridden if the peak is a microvariant allele or an anomaly that has been confirmed.
 - c. PHR – Peak Height Ratio
 - i. May be overridden once it has been confirmed or if the flag was thrown due to an artifact being labeled as an allele or OL. PHR in controls do not have to be verified, provided the genotype is correct.
 - d. MPH – Max Peak Height

- i. May be overridden if any anomalies observed do not affect the quality of the genotyping result.
- e. LPH – Low Peak Height
 - i. May not be overridden for true alleles
- f. AN – Allele Number
 - i. May be overridden in the case of confirmed tri-alleles, no X in Amelogenin (if it is confirmed to not be present), or if the software threw the flag due to artifacts and the analyst evaluated them as such.
 - ii. May not be overridden under any other circumstance.
- g. BD – Broad Peak
 - i. May be overridden if the sample is free from OL designations and the positive controls in the run folder genotyped correctly.
- h. CC – Control Concordance
 - i. May not be overridden unless the software threw the flag due to artifacts and the analyst evaluated them as such.
- i. SPK – Marker Spike
 - i. May be overridden in samples but not ladders.
- j. OVL – Overlapping Alleles
 - i. May be overridden if the allele can be confirmed or in the case of a known artifact.

25.4 START A NEW PROJECT

1. Start with a blank project in the Project Window.
2. Click the Add Samples to Project Icon.
3. Navigate to the location of the desired run folder, then click Add to List.
4. Click Add.
5. Select the desired table view under Table Setting.
6. Ensure the appropriate Sample Type, Analysis Method, Panel, and Size Standard are selected.
7. If a laboratory positive control was used in the run folder, select it from the drop down menu under Custom Control.
8. Click the green arrow to analyze the samples.
9. In the Save Project dialogue box enter the following:
 - a. Name: the project name
 - b. Security Group: Databasing Security Group
 - c. Click OK

25.5 EVALUATION OF LADDERS AND CONTROLS

1. Select the Analysis Summary tab.
2. Review all passing ladders. Any failed ladders must be evaluated by an analyst to verify the failure wasn't due to a factor that affected the corresponding injection.

3. From the Analysis Summary tab, evaluate the passing positive, negative, and custom controls. For failed controls, an analyst may override a flag that has been triggered depending on the type of anomaly.
 - a. Reagent Blank
 - i. No forensically significant PCR product should be detected in the reagent blank sample. See the contamination flow chart for a reagent blank in Appendix II of the DNA Quality Manual should contamination be suspected.
 - ii. The reagent blank must be injected at least as long as the corresponding samples.
 - b. Internal Laboratory Control (Custom Control)
 - i. When used, the internal laboratory control (ILC) should yield the correct allele designations and be free of forensically significant contamination. See the contamination flow chart for a sample in Appendix II of the DNA Quality Manual should contamination be suspected.
 - c. Positive Amplification Control
 - i. The positive amplification control (+C: DNA from the female cell line 9947A) should yield the correct allele designations and be free of forensically significant contamination. See the contamination flow chart for a sample in Appendix II of the DNA Quality Manual should contamination be suspected.
 - ii. Because multiple instances of the positive amplification control could exist on a plate, at least one should pass.
 - d. Negative Amplification Control
 - i. No forensically significant PCR product should be detected in the negative amplification control (-C). See the contamination flow chart in Appendix II of the DNA Quality Manual for a negative amplification control should contamination be suspected.
 - ii. The -C must be injected at least as long as the corresponding +C.

25.6 EVALUATION OF SAMPLES

An analyst must evaluate all samples in a run folder. An analyst may choose to view passing samples separately from samples that did not meet all of the thresholds or an analyst may view all samples together. To view all samples together, select the number under Total # of Samples. To view samples where all thresholds were met, select that number, and to view samples where one or more thresholds were not met, click that number. Work flow is up to the analyst as long as all samples are evaluated.

1. Highlight a sample and select Display Plots.
2. Evaluate the sample to verify allele designations and that the profile is free from anomalies or contamination.
 - a. If more than two true allele peaks occur at multiple loci (two or more), then the profile should be considered as having originated from more than one individual. Occasionally, three alleles may be detected at any one locus. If there is no other indication of more than one source for the DNA it is permissible to assume a single source and accept the genotype result for CODIS after the appropriate confirmations are made.

- b. If contamination is suspected, refer to Appendix II of the DNA Quality Manual for the appropriate troubleshooting steps.
 3. For samples that were flagged by GeneMapper® ID-X, select the locus with the flag and evaluate the description of the flag in the Quality Value Details box.
 - a. If the flag is something an analyst can override, make the appropriate change and “Override the Genotype Quality” at that marker. Appropriate overrides are discussed previously in this section.
 - i. Continue evaluating other flags in the marker; override those that are appropriate to override.
 - ii. When prompted to override the Composite Genotype Quality, select No.
 - iii. Evaluate the remaining non-flagged loci to ensure there are no anomalies or evidence of contamination and that the analyst agrees with the allele calls.
 - b. If a flag is not appropriate to be overridden, continued evaluation of the sample is unnecessary except in terms of troubleshooting what steps (in the laboratory or otherwise) to take next with that sample.
 - c. For all analyst-evaluated passing samples, change the Export Type to the appropriate designation.
 4. Repeat the process for the remaining samples in the project.

25.7 CODIS DATABASE

Profiles deemed appropriate to enter into the state CODIS database and/or NDIS must be peer reviewed prior to entry. Refer to the WSP CLD CODIS Manual for further guidelines on entering convicted offender sample profiles into the state CODIS database and NDIS.

If a sample is appropriate for NDIS or SDIS, make an electronic or manual CODIS table (as appropriate) for that sample.

1. For electronic tables:
 - a. Select the Export Type in the column next to the sample.
 - b. When all appropriate samples to be exported have been selected, choose File > Export Table for CODIS.
 - c. Ensure the appropriate destination and source laboratories are selected and that the file type is CMF 3.2 (.xml).
 - d. The file name for the CODIS table should be the same as the run folder name.
 - e. Choose the appropriate location to save the table and click Export.
2. For manual tables:
 - a. Make a manual table in Microsoft Excel being sure to include all loci and reported alleles.
 - b. For weak samples, single alleles above the analytical threshold and below the stochastic threshold will be in parenthesis. These alleles will not be entered into CODIS, but may be used for exclusion or evaluating matches.

26.0 GUIDELINES FOR EVALUATING CONVICTED OFFENDER DNA TYPING PROFILES USING GENEMAPPER® ID-X V1.4 AS AN EXPERT SYSTEM

26.1 EVALUATION OF SAMPLES USING ID-X AS AN EXPERT SYSTEM

This procedure shall be followed to optimize workflow and expedite data analysis when using GeneMapper ID-X as an Expert System.

26.1.1 Evaluating Ladders and Controls

1. From the Analysis Summary tab:
 - a. Any passing allelic ladders do not have to be evaluated.
 - b. Failed allelic ladders must be evaluated by an analyst to verify the failure wasn't due to a factor that affected the corresponding injection.
 - c. In the case of more than one of the same kind of control (i.e. amplification positive controls that were amplified at the same time on the same thermal cycler), only one iteration need pass. If there is not a positive control under the "All Thresholds Met" column, view the positive control(s) under the "One or more thresholds not met" column and evaluate why they did not pass. Follow the guidelines already described [in this manual](#) for evaluation of positive controls.
 - d. Select the negative controls and verify no trace contamination exists.

26.1.2 Evaluating Samples

An analyst is only required to view samples under the "One or more thresholds not met" column. To maximize workflow, an analyst should not view samples under the "All Thresholds Met" column for routine data analysis.

1. If the project contains samples to be uploaded to CODIS, select the number of the samples under the "All Thresholds Met" column under the "Analysis Summary" tab. Change the "Export Type" of all samples in this column to the appropriate designation. Otherwise, proceed to the next step.
2. From the "Analysis Summary" tab, select the number of the samples under the "One or more thresholds not met" column.
3. Highlight a sample and display the plot. Use a plot setting that allows for the "Quality Value Details" box to be visible.
4. Select the first locus with a flag and evaluate the description of the flag in the "Quality Value Details" box.
5. If the flag is something an analyst can override:
 - a. Make the appropriate change and "Override the Genotype Quality" at that marker. Appropriate overrides are discussed previously [in this manual](#).
 - b. Continue evaluating other flags in the sample; override those that are appropriate to override.
 - c. When prompted to override the "Composite Genotype Quality", select No.
 - d. Evaluate the remaining non-flagged loci to ensure there are no anomalies or evidence of contamination and that the analyst agrees with the allele calls.

6. If a flag is not appropriate to be overridden, continued evaluation of the sample is unnecessary except in terms of troubleshooting what steps (in the laboratory or otherwise) to take next with that sample.
7. If more than two true allele peaks occur at multiple loci (two or more), the profile should be considered as having originated from more than one individual. Occasionally, three alleles may be detected at any one locus. If there is no other indication of more than one source for the DNA profile, it is permissible to assume a single source and accept the genotype result for CODIS after the appropriate confirmations are made.
 - e. If contamination is suspected, refer to [Appendix II of the DNA Quality Manual](#) for the appropriate troubleshooting steps.
8. If an analyst overrode the flags to pass the sample, change the Export Type to the appropriate designation.

Repeat the process for the remaining samples in the “One or more thresholds not met” column.

26.1.3 CODIS Tables

If manual CODIS tables need to be prepared, make those prior to technical review, print the table and include it in the case file.

For electronic CODIS tables, export any samples for CODIS after the technical reviewer has evaluated the run folder. When exporting samples for CODIS tables, select an unfiltered view of the project to ensure inclusion of all exportable samples.

27.0 CODIS CASE FILE CONTENT

A CODIS Case File consists of all documentation related to CODIS Casework including hard copy and electronic data and/or worksheets. A CODIS Case File will contain, at minimum, the following items filled out with sufficient information as to appropriately record all of the pertinent data.

27.1 CODIS CASE FILES

1. Extraction Worksheet (for any manual extractions)
2. 7500 Load Sheet (for any manual 7500 runs)
3. Amplification Worksheet (for any manual amplification set-ups)
4. Robot Worksheet (for any robot sets)
5. Run Folder Review Sheet
6. CE Loading & Reagent Worksheet (if applicable)
7. Manual CODIS Table (when appropriate)
8. CODIS Table (when appropriate)
9. PDF of the puncher work list (when appropriate)
10. Quantifiler™ Projects
11. Normalization File (when appropriate) and Normalization Report (when appropriate)
12. Raw Data folder(s) containing only those samples and controls related to a particular set
13. The GeneMapper® ID-X project(s) for any data that was reported (exported with analysis settings)
14. The peer review checklist (to be added by the technical reviewer)

28.0 MANUAL CODIS CASE FILE REVIEW WITH GENEMAPPER® ID-X V1.4

There are two fundamental steps to the review of CODIS case files which are to be performed by a qualified CODIS scientist:

1. Review of the electronic data.
2. Review of the associated paperwork from extraction through analysis to verify documentation is accurate.

It is the responsibility of the reviewer to ensure the analyzed data is as accurate and complete as possible. At minimum the following will be done:

1. Verify that all electronic documents and files are present.
2. Check the GeneMapper® ID-X results.
 - a. The ladders and quality control samples will be evaluated by the reviewer to ensure they meet the requirements as described in the Guidelines for Evaluating DNA Typing Profiles using GMID-X section of this manual.
 - b. The reviewer must look at all samples (to include the ILS) reported from a run folder.
 - i. For samples where a flag was overridden or an edit was made, the reviewer must evaluate the changes.
 - ii. When an edit has been made, the reviewer must ensure the original analyst documented the change appropriately in the GMID-X software.
 - iii. If the reviewer agrees with the edited profile and/or the analyst's assessment to override flags, the reviewer indicates this by overriding the Composite Genotype Quality (CGQ) of the sample.
 - iv. For passing samples, the reviewer will evaluate the profiles for allele designations and that the sample is free from extraneous peaks.
 - c. All samples shall meet the requirements outlined in the Guidelines for Evaluating DNA Typing Profiles using GMID-X section of this manual. In instances where they do not, but there is cause to report the sample, the CODIS Technical Lead or DNA Technical Leader may sign off on those samples if it is viewed as appropriate. This must be reflected in the case file.
 - d. The CODIS tables will be evaluated to ensure the samples are reported from the correct run folder, the table is the correct export version, each sample has the appropriate Export Type, and that the analyst's CODIS username is correct.
3. Review the paper worksheets.
 - a. Check that all information is filled out on the worksheets and that the appropriate worksheets are present.
 - b. Check that the case numbers of the convicted offender samples are accurate across all worksheets.
 - c. Verify that a set number, date, and initials are on each page.
 - d. Ensure that all strikeouts and additions are initialed.

For a more detailed list of what is required for the technical review of a CODIS Case File, see the CODIS technical review worksheet.

29.0 CODIS CASE FILE REVIEW WITH GENEMAPPER® ID-X V1.4 AS AN EXPERT SYSTEM

29.1 TECHNICAL REVIEW OF GENEMAPPER ID-X AS AN EXPERT SYSTEM

Open the first project in GeneMapper ID-X.

29.1.1 Review of ladders

Ladders do not have to be evaluated by a technical reviewer.

29.1.2 Review of controls

The ILS of a control under the “All Thresholds Met” column does not have to be reviewed by the technical reviewer.

From the Analysis Summary tab:

1. Check the negative controls under the “All Thresholds Met” column for trace contamination.
2. Do not look at the other controls under the “All Thresholds Met” column.
3. If any controls are under the “One or more thresholds not met” column, click on the number under the total number of controls in that column.
4. Look at any control reported from the project to verify analyst overrides are appropriate.
5. For any control with allele edits, use the “View Allele Edits” plot setting to verify artifacts/alleles are documented appropriately.
6. If in agreement with the control overrides/edits, override the CGQ of the control.
7. For projects with more than one positive amplification control from the same amplification, only one of those controls must pass.

29.1.3 Review of samples

The ILS of a sample under the “All Thresholds Met” column does not have to be reviewed by the technical reviewer.

1. Do not view the genotypes of samples under the “All Thresholds Met” column.
2. Click on the number under the “One or more thresholds not met” column.
3. Look at any sample reported from the project to verify analyst overrides are appropriate.
4. For any sample with allele edits, use the “View Allele Edits” plot setting to verify artifacts/alleles are documented appropriately.
5. If in agreement with the sample overrides/edits, override the CGQ of the sample.
6. Continue for all reported samples from the run folder.

Continue reviewing the case file and other projects, if applicable.

For confirmation runs, it is appropriate to review the original iteration of the sample to verify an appropriate confirmation was made.

Review the electronic CODIS tables to ensure the appropriate samples are reported from the correct run folder with the correct Export Type, export version, and analyst CODIS username.

For additional details on the requirements of technical review, see the [CODIS Peer Review Checklist](#).

30.0 TEMPGENIUS™ WIRELESS DATA ACQUISITION & MONITORING SYSTEM

The TempGenius system is used to wirelessly record the temperature for refrigerators, freezers, and ovens in the lab. Rather than recording the temperature of a unit at one time of day, TempGenius constantly monitors and records the temperature. If a unit falls outside of the desired temperature range, TempGenius will alert the user and record the event. This allows for more effective monitoring of refrigerators, freezers, and ovens.

30.1 GENERAL INSTALLATION AND OPERATION

Refer to the TempGenius “Installation Guide And Operating Manual”.

30.2 ALERTS AND RESOLUTIONS

TempGenius automatically monitors temperatures and sends alerts to the assigned user(s) and TempGenius Dashboard. In addition to viewing temperatures on the Dashboard, real-time temperatures may be viewed remotely by accessing the IP address of the TempGenius Data Server. Refer to the TempGenius Installation Guide and Operating Manual for instructions on alerts and alert resolutions.

30.3 TROUBLESHOOTING ALERTS

Use the following table as a guide for troubleshooting alerts.

Alert Type	Critical Unit?	Action	Address Off-hours?
Level 1	No	If more than two days in a row, verify reading against a NIST thermometer. If NIST is within allowable range, contact the TempGenius rep, otherwise troubleshoot until a solution is reached.	No
Level 1	Yes	If more than two days in a row, verify temperature reading against a NIST thermometer. If NIST is within the allowable range, contact the TempGenius rep, otherwise move contents of unit to another location until a solution can be reached.	No
Level 2	No	Verify reading against a NIST thermometer. If NIST is within allowable range, contact the TempGenius rep, otherwise troubleshoot until a solution is reached.	No
Level 2	Yes	Verify the temperature reading against a NIST thermometer. If NIST is within the allowable range, contact the TempGenius rep, otherwise move contents of unit to another location until a solution can be reached.	Yes, if a volunteer is unavailable, notify CODIS Manager
Level 3	No	Verify the temperature reading against a NIST thermometer. If NIST is within the allowable range, contact the TempGenius rep, otherwise troubleshoot as needed.	No
Level 3	Yes	Verify the temperature reading against a NIST thermometer. If NIST is within the allowable range, contact the TempGenius rep, otherwise move contents of unit to another location until a solution can be reached.	Yes, if a volunteer is unavailable, notify CODIS Manager

31.0 REAGENT PREPARATION

Use reagent grade chemicals unless otherwise noted. Pre-made, reagent grade chemicals can be substituted for many of the recipes below. Prepare all solutions using deionized water (dH₂O). Wear gloves and follow safety recommendations provided by manufacturer for handling chemicals. Comply with any and all laws, regulations, or orders with respect to the disposal of any hazardous or toxic chemical, material, substance or waste. Reagents that are used solely for the preparation of other reagents do not need to be sterilized before making up the new reagent if that reagent will be sterilized. Store all reagents at room temperature unless otherwise noted. Reagents will be labeled with the reagent name, lot number, expiration date (if any), preparer's initials, and date of preparation. Reagents are prepared as follows:

31.1 0.5M EDTA (1 L)

Warning: EDTA is an irritant. Wear lab coat, gloves, mask, and protective eyewear when handling EDTA.

Warning: NaOH is corrosive and toxic. Wear lab coat, gloves, and protective eyewear. Use caution when handling. NaOH, when combined with water, results in an exothermic reaction. Dissolve the NaOH pellets in the water gradually with 3 or 4 additions. Cover and allow the NaOH to dissolve completely and cool between each addition.

Add 186.1 g of disodium ethylenediaminetetraacetic acid dihydrate (Na₂EDTA · 2H₂O) to 800 ml of dH₂O. Stir vigorously with a magnetic stirrer. To dissolve the EDTA powder, adjust the pH to 8.0 (+/- 0.2) by adding approximately 20 g of NaOH pellets. Check the pH and add 5N or 10N NaOH solution if needed. Adjust volume to 1 liter with dH₂O and mix thoroughly. The solution should be autoclaved or filtered through a sterile 0.2 µm filter.

31.2 TE BUFFER [10 MM TRIS-HCL, 0.1 MM EDTA, PH 8.0 (1 L)]

Add 10 ml of 1 M Tris-HCl, pH 8.0 and 0.2 ml of 0.5 M EDTA to 990 ml dH₂O and mix thoroughly. Autoclave.

31.3 1 M TRIS-HCL, PH 8.0 (1 L)

Dissolve 98 g Tris-HCl and 46 g Tris base in 800 ml of dH₂O. Check that the pH of the solution is 8.0 (+/- 0.2). Adjust final volume to 1 liter with dH₂O and mix thoroughly. Sterilize by autoclaving.

31.4 1 M DITHIOHREITOL (DTT - 10 ML)

Add 1.542 g of DTT to 10 ml dH₂O. Aliquot 1 ml of solution to ten tubes. Store frozen.

31.5 LYSIS BUFFER + DTT

Prepare Lysis Buffer + DTT by mixing 1 ml of 1.0 M DTT for every 100 ml DNA IQ™ Lysis Buffer. Mix by inverting several times. Store at room temperature.

31.6 1X WASH BUFFER

Prepare 1X Wash Buffer by mixing one part 95-100% ethanol, one part isopropyl alcohol, and two parts DNA IQ™ 2X Wash Buffer. Mix by inverting several times. Store at room temperature.

31.7 QUANTIFILER™ QUANTIFICATION STANDARDS

DNA quantitation standards are critical for accurate analysis of run data. Any mistakes or inaccuracies in making the dilutions directly affect the quality of the results. Great care shall be taken when measuring and mixing dilutions.

1. Prepare the DNA Quantitation Standards using G147A (Promega catalog #G1471) genomic DNA (SRM 2372 traceable)
2. Label eight disposable tubes to be used for the dilution series.
3. Prepare the TE with glycogen by adding 1µl glycogen to 1 ml of TE. Vortex.

4. Dispense the required amount of TE/glycogen to each tube. (See note* on next page to adjust for lot to lot variation in stock DNA standard.)
5. Prepare the standards using the following table.
 - a. For standard 1:
 - i. Briefly vortex the G147A Human DNA Standard and pulse spin.
 - ii. Using a new pipette tip, add the calculated amount of G147A Human DNA Standard to the tube for Standard 1.
 - iii. Vortex and pulse spin the dilution
 - b. For standards 2 through 8:
 - i. Using a new pipette tip, add the calculated amount of the previously prepared standard to the tube for the next standard.
 - ii. Vortex and pulse spin the standard.
 - iii. Repeat steps d.2.i and d.2.ii until the dilution series is completed.
6. The prepared Human DNA Standards may be stored for up to 4 weeks at 2 to 8°C.

Standard	Concentration (ng/ul)	Example Amounts	Minimum Amounts†	Dilution Factor
Std. 1	50.000	50 µl [200 ng/µl stock] + 150 µl TE/glycogen buffer	10 µl [200 ng/ul stock] + 30 µl TE/glycogen buffer	4X
Std. 2	16.700	50 µl [Std. 1] + 100 µl TE/glycogen buffer	10 µl [Std. 1] + 20 µl TE/glycogen buffer	3X
Std. 3	5.560	50 µl [Std. 2] + 100 µl TE/glycogen buffer	10 µl [Std. 2] + 20 µl TE/glycogen buffer	3X
Std. 4	1.850	50 µl [Std. 3] + 100 µl TE/glycogen buffer	10 µl [Std. 3] + 20 µl TE/glycogen buffer	3X
Std. 5	0.620	50 µl [Std. 4] + 100 µl TE/glycogen buffer	10 µl [Std. 4] + 20 µl TE/glycogen buffer	3X
Std. 6	0.210	50 µl [Std. 5] + 100 µl TE/glycogen buffer	10 µl [Std. 5] + 20 µl TE/glycogen buffer	3X
Std. 7	0.068	50 µl [Std. 6] + 100 µl TE/glycogen buffer	10 µl [Std. 6] + 20 µl TE/glycogen buffer	3X
Std. 8	0.023	50 µl [Std. 7] + 100 µl TE/glycogen buffer	10 µl [Std. 7] + 20 µl TE/glycogen buffer	3X

† To ensure the accuracy of pipetting, the minimum input volume of DNA for dilutions is 10 µl.

***NOTE: How to adjust for lot to lot variation in the Human DNA Standard:**

Use the SRM 2372 kit from NIST to determine the DNA concentration of each new lot of G147A DNA standard. For step 1 of preparing the Quantitation Standard sets, dilute the stock according to the actual concentration obtained for the new lot of DNA. The rest of the dilution steps remain the same.

31.8 DISPENSING HI-DI FORMAMIDE

Hi-Di Formamide is harmful if swallowed, inhaled, or absorbed through the skin. See MSDS for full disclaimer. When aliquoting Hi-Di Formamide, work in a fume hood with gloves and lab coat.

Remove the Hi-Di bottle from the freezer and allow it to come to room temperature until contents have thawed. Aliquot 1 mL Hi-Di into 1.5 mL autoclaved tubes. Label each tube with the lot, date of dispensing, and initials. Freeze immediately.

31.9 REAGENT EXPIRATION DATES

The following table lists expiration dates for reagents used in the CODIS Laboratory. Reagents that are tested before each use do not require an expiration date. Should a stock solution purchased from a distributor be used rather than being made in the lab, the expiration date on the bottle overrides any expiration date listed here.

Commercially prepared critical reagents that do not have an expiration date assigned by the manufacturer will be assigned one by the laboratory based on performance testing and review of scientific literature or up to three years from receipt.

Non-critical commercially prepared reagents that do not have an expiration date assigned by the manufacturer do not need one assigned by the laboratory provided they are stored and maintained appropriately. The integrity of these reagents, as with any reagent, will be monitored at the time of use.

Reagent Expiration Dates

DTT	3 years (stored frozen)
0.5 M EDTA	3 years
TE Buffer	2 years
1M Tris-HCl	3 years
Lysis Buffer + DTT	1 month
1X Wash Buffer	1 year
QF Standards	4 weeks if in glycogen

32.0 TEMPGENIUS™ WIRELESS DATA ACQUISITION & MONITORING SYSTEM MAINTENANCE

32.1 SEMIANNUALLY

A NIST probe reading for each monitored unit will be recorded semiannually, to ensure the accuracy of the temperature readings recorded by TempGenius. If the difference between the TempGenius reading and the NIST reading is outside the allowable range, another reading will be taken at a different time. In the event that the TempGenius reading is once more outside the range, contact the manufacturer.

For Daylight Savings time changes, a “write setup” from the Pointware program to the data receiver must be performed to sync the computer and device time. Before performing the write setup, ensure that the historical data collections are current. This can be done by making sure the time of “last contact” listed in Pointware (under the Point Managers tab) is the same as the current time on the computer.

Once the historical data are current, perform the write setup in Pointware via the Point Managers tab.

1. Right-click on the appropriate Point Manager name
2. Select “Push”
3. Select “Write Setup”
4. Select “Yes”.

32.2 ANNUALLY

Calibration of sensors – see the TempGenius “Installation Guide and Operating Manual”

The manufacturer defines the annual calibration as the adjustment (or replacement) of a sensor due to temperature variability against a NIST probe. The average offset for a sensor will be determined based on the total readings for the year. The laboratory may choose to make an adjustment if the offset between the sensor and NIST probe is +/-1°C. If the average offset of a sensor is more/less than +/- 4°C, an adjustment is mandatory and the manufacturer will be contacted.

If any adjustment is made to the sensor, a reading with A NIST probe will be taken the following day to verify the adjustment. All adjustments will be documented on the TempGenius maintenance sheet.

32.3 EVERY 30 MONTHS OR AS NEEDED

Replace the batteries (Type AA, 3.6V) in the sensors by unscrewing the transmitter cover.

33.0 UV IRRADIATOR OPERATING INSTRUCTIONS

33.1 OPERATION

1. Turn the power on. The digital display should read 0000 or 0; if it does not, press reset.
2. Place the consumables in an orientation to allow maximum exposure.
3. Select the Energy button. The number on the display represents microjoules/cm² x 100.
 - For autoclaved consumables, set the microjoule level to 500,000 $\mu\text{J}/\text{cm}^2$.
 - For non-autoclavable consumables, set the microjoule level to 999,900 $\mu\text{J}/\text{cm}^2$
4. If an error is made in entering, press the reset button to clear the display.
5. Press start to begin irradiation.

33.2 MAINTENANCE

1. Clean the floor of the UV Irradiator with a soft cloth and nonabrasive cleaner once a week.
2. Change bulbs as needed.

34.0 WALLAC DBS PUNCHER; INSTRUMENT MAINTENANCE

34.1 WEEKLY

1. Clean the instrument cover and plate area
 - a. Clean the instrument cover and plate area with a soft cloth or Kimwipe[®] tissue that has been sprayed with a mild detergent solution or alcohol.
 - b. Clean the plate area with an anti-static device.

34.2 ANNUALLY

Instrument maintenance can be performed by a qualified Perkin Elmer representative or by the University of Washington's Scientific Instruments Service Center.

34.3 AS NEEDED

1. Replace the puncher piston and punch counter
 - a. The puncher piston and punch counter should be replaced after 400,000 punches.
 - b. The punch count can be determined through the display menu under configuration.
 - c. Replace the piston and reset the counter following manufacturer's operating manual. This can be included as part of the manufacturer's scheduled service maintenance.

35.0 QIAGEN BIOROBOT UNIVERSAL MAINTENANCE

When a maintenance procedure is due, the “Run” icon in the Execute Environment will be yellow. Selecting the Maintenance Environment will allow the user to view the current maintenance procedures that are due and all maintenance procedures available. Detailed instructions are provided for each procedure in the Maintenance Environment.

Because the BioRobot Universals are not necessarily used on a daily basis, maintenance protocols that are due on a more frequent basis than the robot is used will be run before each use rather than by time. The exception to this is the Preventive maintenance procedure that is conducted by a qualified technician.

Certain maintenance procedures listed in the BioRobot Universal Manual are for features of the robot not used by the CODIS Laboratory. Those maintenance procedures are not required.

35.1 DAILY MAINTENANCE PROCEDURES

Perform the following daily maintenance procedures:

1. Liquid containers
2. Tip-disposal station
3. Worktable

35.2 WEEKLY MAINTENANCE PROCEDURES

Perform the following weekly maintenance procedure:

1. Reagent carousel

35.3 MONTHLY MAINTENANCE PROCEDURES

The weekly and daily maintenance will be completed before starting any monthly maintenance.

Perform the following monthly maintenance procedures:

1. System liquid container
2. High-speed dispensing system and liquid detectors
3. Worktable
4. Robotic handling system

35.4 BIENNIAL MAINTENANCE

1. Check the dilutor system
 - a. From the Execute Environment, select Syringe Calibration Check UNIV from the protocol selection box and click Run. Follow the wizard instructions.
 - b. If the dilutor system needs to be calibrated, select Syringe Calibration Adjust UNIV from the protocol selection box and click Run. Follow the wizard instructions.

35.5 ANNUAL MAINTENANCE

Annual preventive maintenance is performed by a qualified technician and includes a check of the dilutor system.

36.0 BIOTEK PLATE READER – INSTRUMENT MAINTENANCE

36.1 BEFORE USE:

1. Turn off and disconnect the instrument from the power supply.
2. Moisten a clean cotton cloth with water or with water and a mild detergent – do not soak cloth.
3. Wipe the plate carrier and all exposed surfaces of the instrument.
4. If detergent was used, wipe all surfaces with a cloth moistened with water.
5. Use a clean, dry cloth to dry all wet surfaces.

36.2 MONTHLY

1. Run the Absorbance Plate Test
 - a. Follow the procedure for the Absorbance Plate Test as outlined in the BioTek plate reader user manual.

36.3 SEMI-ANNUALLY

1. Run Liquid Test 1
 - a. Follow the procedure for Liquid Test 1 as outlined in the BioTek Plate reader user manual.

36.4 AS NEEDED:

1. Replace the bulb
 - a. Follow the procedure for bulb replacement as outlined in the BioTek Plate reader user manual.

36.5 ANNUALLY:

1. The Absorbance Test Plate shall be sent out for re-certification.

37.0 ABI 7500 – INSTRUMENT MAINTENANCE

See the ABI 7500 Instrument Maintenance manual for specific instructions:

37.1 ON A MONTHLY BASIS:

1. Check sample block for contamination
2. Perform a background calibration
3. Monitor the lamp status
4. Perform a Function-Test

37.2 SEMI-ANNUALLY

1. Perform an Optical calibration
2. Perform an ROI calibration
3. Perform a Pure Dye Spectra calibration

37.3 IF THE INSTRUMENT IS MOVED TO A NEW LABORATORY LOCATION, THE FOLLOWING SHALL BE PERFORMED:

1. ROI calibration
2. Pure Dye Spectra calibration

37.4 ON AN AS-NEEDED BASIS OR DURING SCHEDULED DATA ARCHIVAL, THE FOLLOWING SHALL BE PERFORMED:

1. Back up files
2. Run disk cleanup
3. Clean sample wells
4. Replace instrument fuses
5. Replace the halogen bulb
6. The RNase P verification plate may be run as necessary for troubleshooting purposes.

NOTE: The RNase P plate is one time use only; other maintenance plates are re-useable until the plate warps.

After approximately 2000 hours of use, the bulb shall be replaced. When the bulb is replaced, the following shall be performed:

1. Calibrate the ROI
2. Run a background calibration
3. Perform an Optical Calibration
4. Run the Pure Dye calibration
5. Verify the Instrument Performance

If a hardware problem is suspected, a Function Test may be run for troubleshooting purposes. A record of the checks shall be kept in each instrument log. Retention of the monthly maintenance shall be for a minimum of one (1) accreditation cycle.

When a new 7500 instrument is installed, a performance verification plan addressing sensitivity and precision will be drafted and submitted to the DNA Technical Leader for approval. The results of the performance test will be summarized and retained in the instrument log book.

38.0 9700 THERMAL CYCLER – INSTRUMENT MAINTENANCE

38.1 BIANNUALLY

Consult with the manufacturer's recommendations when running the following tests:

Run the Calibration Verification Test

Run the Temperature Non-Uniformity Test Overview

38.2 AS NEEDED

Send to Instrument Services for external calibration

39.0 3500XL GENETIC ANALYZER MAINTENANCE

Spatial Calibration

A spatial calibration must be performed every time a capillary array is removed or replaced, the detector door is opened, or the instrument is moved.

1. In the Maintenance navigation pane, select 'Spatial Calibration'.
2. Select either 'Fill' or 'No Fill', then start the calibration.
3. Evaluate the calibration results to ensure that there is:
 - a. A sharp peak for each capillary (small shoulders are acceptable).
 - b. A '+' marker at the apex of each peak and no off-apex markers.
 - c. An even peak profile (all peak about the same height).
4. Accept the results if the criteria above are met. Refer to the 3500xL User Guide if the calibration fails.

Spectral Calibration

Perform a spectral calibration when a new dye set/polymer combination is used, if a new capillary array is installed, after any service on the optics, or if there is an increase in pull-up observed.

1. Briefly vortex and spin the DS-33 Spectral Standard. Prepare a solution by mixing 3 μ L DS-33 with 297 μ L Hi-Di Formamide. The standard may be diluted more if the peaks are too saturated.
2. Load 10 μ L of the standard solution into columns 1 – 3 of a plate. These columns MUST be used. Cover the plate with a septum and briefly spin to remove any bubbles.
3. Denature samples on a 95°C heat block or thermal cycler for 5 minutes, then snap-cool for 3 minutes.
4. Place the assembled plate onto the instrument. Select 'Spectral Calibration' in the Maintenance navigation pane and verify the calibration settings.
 - a. The software will automatically run up to 3 injections to obtain a passing calibration.
 - b. Not allowing capillary borrowing generally produces better results, but the software may be configured to allow up to 3 borrowing events.
5. Start the calibration.
6. Evaluate the results and choose 'Accept' or 'Reject'. Refer to the 3500xL User Guide for detailed instructions on evaluating calibration data.

Maintenance

The 3500 Series Software employs wizards to assist the user in performing maintenance procedures. Access the appropriate wizard from the Maintenance Wizard screen and follow the prompts to complete any required tasks. The Maintenance calendar on the Dashboard may also be used to remind users when task are due.

Before each run

- Check for any bubbles in the pump block and lines. If present, use the Remove Bubble Wizard.
- Check consumables on the Dashboard. Replace polymer and buffers if needed.

Weekly

- Restart the instrument and computer.

CODIS Lab STR Procedures	Page 71 of 87	Revision June 1, 2016
Approved by CLD Commander	All Printed Copies Uncontrolled	Revision 22

- Clean the anode buffer container valve pin.

Monthly

- Run the Wash Pump and Channels wizard and flush the pump trap.
- Replace the cathode buffer container septa.
- Clean the autosampler and drip tray.

Quarterly

- Run the HID install standard performance check (refer to the 3500xL User Guide).

Annually

- Preventive maintenance will be performed by a qualified technician.

As needed

- Replace the capillary array using the Install Capillary Array wizard when poor precision or allele calling is noted, or when resolution or signal intensity decreases. Arrays may be used past the manufacturer's expiration date.
- Perform spectral or spatial calibrations (see sections above).
- If the instrument will not be in use for more than 2 weeks, refer to the 3500xL User Guide for further information on proper care of the instrument.

Performance Checks

A performance check will be done on an instrument if it has been moved and following repair or routine maintenance done by a qualified technician. The check must be completed prior to use for convicted offender sample analysis and consists of successfully running:

1. A ladder, a negative amplification control, and a positive amplification control (or other known sample) and
2. If the sensitivity of the instrument may have been affected (e.g. the optics were adjusted), the HID install standard performance check

For new instruments, a 1 base pair precision check with at least 6 ladders run per capillary is required in addition to the performance check detailed above.

40.0 MAINTENANCE PROCEDURES – GENEMAPPER® ID-X V1.4 SERVER VERSION ONLY

40.1 WEEKLY

1. Exit the software and re-start the computer
2. Review the statistics for the database (see the GeneMapper ID-X Administrator's Guide for the full procedure)

40.2 QUARTERLY

Run calibration samples according to the NDIS Procedures for Expert Systems

40.3 AS NEEDED

1. Review password policies
2. If using the audit system, review
3. If using the electronic signature system, review
4. Manage user accounts
5. Manage security groups
6. Manage profiles
7. Back up security settings after making changes
8. Back up and delete audit trail records when records are close to or exceed 40,000 records.

41.0 TUTTNAUER TABLETOP AUTOCLAVE – INSTRUMENT MAINTENANCE

41.1 PRIOR TO USE

1. Fill the water reservoir
 - a. Remove the water reservoir cover.
 - b. Pour deionized water into the reservoir, through the opening on the top of the autoclave until it reaches the base of the safety valve holder (approximately 3 liters).

41.2 WEEKLY

1. Clean the door gasket
 - a. Clean the door gasket with a mild detergent, water and a soft cloth or sponge. The gasket should be clean and smooth.

41.3 MONTHLY

1. Change the water in the water reservoir
 - a. Ensure that the electrical cord is disconnected and there is no pressure in the autoclave.
 - b. To drain the reservoir, turn the drain valve counter clockwise to the open position. Place a bucket underneath to catch the flow of water. Fully drain the reservoir.
 - c. With a quart of tap water flush out the reservoir.
 - d. Turn the drain valve clockwise to the closed position and fill the reservoir with deionized water to just below the safety valve. Plug the electrical cord in.
2. Clean the water sensor
 - a. Wipe the sides and tip of the water sensor (located in the rear of the chamber) with a mild soapy solution.
 - b. It is very important the sides of the sensor are wiped clean.

41.4 QUARTERLY

1. Clean the air jet
 - a. Remove the water reservoir cover.
 - b. Clean the hole of the jet by manipulating the air trap wire back and forth ten (10) times.
 - c. It is preferred that the cleaning is done while the unit is running a cycle and under pressure, but it can also be done while the unit is idle.
2. Clean and descale the chamber, copper tubes, and reservoir using ChamberBrite™

Follow the procedure for cleaning the autoclave as outlined in the Tuttnauer Tabletop Autoclave maintenance manual.
3. Clean the trays and tray holder

- a. Clean the tray holder and trays with detergent or non-abrasive stainless steel cleaner and water.
- b. Rinse immediately to avoid staining the metal.

41.5 AS NEEDED (SEE PROCEDURES AS OUTLINED IN THE TUTTNAUER TABLETOP AUTOCLAVE MAINTENANCE MANUAL)

- 1. Clean and check the safety valve
- 2. Replace the door gasket
- 3. Clean the fan grid with compressed air from the inside outward
- 4. Clean the strainer
- 5. Inspect the locking device for wear

42.0 APPENDIX A ADMINISTRATIVE PROCEDURES FOR PROCESSING OFFENDER SAMPLES

42.1 RECEIVING SAMPLES

42.1.1 Blood samples:

1. Check that the information on the data submission sheet is complete and matches that on the blood vial. Fill out “Received By” and “Date and Time Rec’d” sections.
2. See the [Processing of Convicted Offender Samples](#) section of this manual for more information about how blood samples are received and stored.

42.1.2 Buccal samples:

1. Group sample envelopes by date received.
1. Group sample envelopes by submitting agency. If there is not a return address, group by postmark and open to determine agency. If there is no postmark, open envelope to determine agency.
2. Group sample envelopes by initials on the security seal.
 - a. If the seal is absent or tampered with, set the sample aside.
 - b. If the initials are absent, open envelope to verify that collector information is filled out. If not, set the sample aside.
3. Open envelopes one at a time keeping the groups together. Verify:
 - a. Offender information is filled out correctly, and a Washington State Identification (SID) Number is present and there is a qualifying offense listed on the submission card.
 - b. Collector information is complete:
 - i. Collector’s signature is present.
 1. If no signature is present, initial and date the “Collector’s Signature” field after verifying that the collector has initialed the security seal on the envelope.
 2. If neither signature nor initials are present, set the sample aside and then call to verify collector.
 - ii. The agency name is present and distinctive.
 1. If the agency is not listed or the abbreviation is ambiguous and it can be determined from: the postmark, return address on the envelope or group of samples it was submitted with; fill in the appropriate information and initial and date the addition.
 - iii. If the collector information cannot be determined, set the sample aside and call for clarification.
 - c. The thumbprints are distinctive and the ridges can be clearly seen.
 - d. The DNA sample is obvious on the FTA[®] paper, i.e. pink paper has turned white.
 - e. For the multi-component kit, verify the convicted offender’s name, SID number (an eight-digit number) and date of birth, if printed on the outer part of the FTA[®] card, corresponds to the information on the submission card.

- f. If criteria in 4.a-4.e are met, skip to the [Assigning Lab Numbers](#) section below.
 - g. If 4.a is insufficient, look up the missing data on the convicted offender's criminal history or call the collecting agency for clarification. If an offense is determined to be non-qualifying, and the offender does not have a previous qualifying conviction, the sample must be rejected.
4. Agencies that submitted samples that were set aside because of a violation in step 3, 4.b.i, or 4.c-4.e should be contacted to request a recollection; emphasize that the entire kit needs to be redone. If a recollection is not possible, the sample may be accepted at the discretion of a CODIS staff member.
 5. When a sample is rejected, fill out the rejection log with the pertinent information.

42.1.3 Assigning Lab Numbers

1. Lab numbers depend upon lab location, the year of submission and the block of numbers assigned to the convicted offender program for that year by the CODIS Manager.
 - a. An example of a lab number assigned to a convicted offender is 103-015234.
 - i. The lab number begins with the lab code (Seattle = 1).
 - ii. The next two digits refer to the year (03 = 2003).
 - iii. The remaining six digits are the actual sample number; their start point is determined by the CODIS manager. Each successive sample is assigned the next number in the sequence.
2. For blood samples, based on the numbering rules above, write the next lab number, in sequential order, in the upper right corner of the submission sheet in the box labeled "Laboratory No."/"Crime Lab Use Only".
3. The remaining steps are for buccal samples. Begin with the presorted samples from the Receiving Samples section.
 - a. For the all-in-one sample card:
 - i. Number the card with the next available number in the sequence in the box labeled "for Crime Lab Use Only". Numbering may be done by hand or with pre-printed labels.
 - b. For the multi-component kit:
 - i. Number a 3³/₈" x 6" clasp envelope with the next available number in the sequence at the top center of the envelope (the envelope flap should be to the left). Numbering may be done by hand or with pre-printed labels.
 - ii. Place the FTA card in the envelope.
 - iii. Number the corresponding submission card on the front, upper-right corner of the folded submission card.
4. Repeat with the remaining samples. Be sure to keep the numbers in order. It is acceptable to intermix both types of buccal collection kits.

42.1.4 Entering Data into the Convicted Offender Entry Form

1. For both blood and buccal samples, open Convicted Offender Entry Form (the user name and password are the same as for LIMS).
2. Make sure Caps Lock is on.

3. Select the proper entry type. When entering an all-in-one sample card, be sure to keep the FTA[®] paper covered.
 - a. Washington State Identification numbers (SIDs) are usually eight digits. When “SID Number” is selected, the entry field will contain a “WA” prefix.
 - b. If a SID number is unavailable, use the “Other Number” type and preface the number with one of the following abbreviations:
 - i. Juvenile numbers are six digits and may be categorized as JUVIS, JRA or JV.
 1. If JUVIS is specified, use JUV.
 2. If JRA is specified, use JRA.
 3. If nothing is specified, but juvenile can be determined by date of birth or facility, then use JV.
 - c. Social Security numbers should be abbreviated as SSN and entered without hyphens or spaces. Verify with the institution beforehand that this is the only ID available.
 - d. Department of Corrections numbers should be abbreviated as DOC. Verify with the institution beforehand that this is the only ID available.
 - e. Local Identification numbers should be abbreviated as Local ID. Verify with the institution beforehand that this is the only ID available.
 - f. Out-of-state Identification numbers should be prefaced with the state’s two letter abbreviation. Verify with the institution beforehand that this is the only ID available.
 - g. FBI numbers shall be prefaced with FBI. Verify with the institution beforehand that this is the only ID available.
 - h. If no number is available indicate “No ID”. Make all attempts to obtain a number from the submitting agency before this step.
4. Click Enter.
5. If using entry type 3.a, the “Convicted Offender Sample Entry” dialogue box will appear containing the name, date of birth, and DNA status found in W2 for the entered SID number. If an error message appears, check the SID to verify that it was entered correctly and if it still does not work, set the sample aside to be looked up later.
 - a. Verify that name and date of birth match the submission card. Some offenders will have multiple names and/or dates of birth; the Convicted Offender Program will insert the first listed name or date of birth in W2 into the Convicted Offender Entry Form. For the sake of consistency, if there is more than one name and/or date of birth listed in the dialogue box, the first one in the list will be assigned to that SID number. If the submission card has an alias name and/or date of birth, note this on the card and write in what will be used (date and initial). If either the name or date of birth on the submission card is not listed in the dialogue box, set the sample aside to be looked up later.
 - b. Check DNA status.
 - i. If it says “DNA NOT Taken”, Click OK.
 - ii. If it says “DNA Taken” stamp the following with a “Duplicate” stamp:

1. For blood samples, stamp the top of the submission sheet and outside of the zip top bag containing the blood sample.
 2. For the all-in-one sample card, stamp the collector information side of the card.
 3. For the multi-component kit, stamp the front center of the clasp envelope and the upper left corner of the open submission card.
- iii. Click OK.
- c. “Last Name”, “First Name”, “Middle Initial” (if available in W2), “Date of Birth”, and “Individual Type” fields will populate automatically. Continue entry as follows:
- i. Select Gender.
 - ii. Tab to “Offense Description”. Enter offense from submission card. Common abbreviations are okay.
 - iii. Tab to “Offense Category”. Select type of offense from drop down box.
 - iv. Tab to “Collector’s Institution”. Enter institution from submission card.
 - v. Tab to “Collector’s Name”. Select the representative that did the collection. If a particular representative is not on the list, they may be added by clicking on the green plus (+) button.
 1. Take off the Caps Lock.
 2. Enter collector’s last name, first name, and any other known collector information in the available fields.
 3. Type your initials and date added in the “Added by” field.
 4. Click OK. (Make sure Caps Lock is back on).
 - vi. Tab to “Request Date”. Enter date received.
 - vii. Verify appropriate barcode quantity is selected (the default is three).
 1. For blood samples, change this to 0.
 2. For the all-in-one kit, change this to 1.
 3. For the multi-component kit, leave as three.
 - viii. Click OK. (Labels will print after the lab number has been entered.)
 - ix. Case ID dialogue box will appear. Enter Lab Number previously assigned.
 - x. Click OK to complete the case entry and print barcode label(s).
6. If using entry type 3.b or 3.c, a new entry form will appear (“Individual Type” will be automatically set). Enter first and last name, middle initial, and date of birth. Continue as in steps 5.c.i – 5.c.x.

42.1.5 Affixing Labels

1. Make sure the number on the label is the same as the number on the Submission Card; and for the multi-component kit, on the corresponding envelope.
2. Place a label on the “CRIME LAB USE ONLY” space on the Submission Card.
3. For the multi-component kit:
 - a. Verify that any information written on the FTA[®] card matches the corresponding Submission Card.

- b. Place the next label on the outer cover of the corresponding FTA[®] card. Do not cover any convicted offender information that may be written on the flap; instead place the label on the back.
- c. Place the last label over the number already present on the outside of the clasp envelope.
- d. Place the FTA[®] and Submission Cards into the envelope.

4. File the sample in numerical order in the filing cabinet.

42.1.6 Administrative Errors

Error	Description	Correction
Duplication of a case number	A particular case number is assigned to more than one sample.	LIMS will not allow duplicate entries of a case number. Upon discovery of such a duplicate, merely assign the next available case number to the submission card and sample envelope (initial if making a strikeout). E-mail the LIMS Administrator with a request to delete the incorrect number.
Mis-entering a case number	A case number is entered into the Convicted Offender form in the wrong format (e.g. 406-123456 or 1061-23456).	Wait for a response that the incorrect number was deleted from LIMS (otherwise a duplicate entry will be created). Ensure that the DNA Taken flag has been changed to no (unless the sample was already a duplicate). Re-enter the sample information with the correct number. Print and affix new labels.
Missing FTA card	An FTA card is not included with its corresponding submission card.	Set the card and envelope aside until the correct pairing can be established. If a match cannot be made, a request will be made of the agency to obtain another sample. Set the card/sample aside until the mismatch is ameliorated.
Switched FTA cards	An FTA card is incorrectly paired with a submission card.	Set the card and envelope aside until the correct pairing can be established. If a match cannot be made, a request may need to be made of the agency to obtain another sample. Set the card/sample aside until the mismatch is ameliorated.
Skipped case number	A case number is omitted in the sequence (e.g. 106-123456 → 106-123458), usually discovered by browsing stored submission cards.	Ensure that the missing number has not previously been assigned to a card that may have been removed from storage. Assign a new card & sample with the skipped case number. This should only be done for samples received in the same year.
Entering the wrong request date	The incorrect request/receipt date was entered into the Convicted Offender entry program.	Wait for the date to be updated in LIMS (usually a day after entry), then manually make the correction. Print and affix new labels.
Mislabeled	A submission card and/or sample envelope is labeled incorrectly.	If possible, remove the incorrect label(s) and determine to which card/envelope they belong. Affix to the appropriate card/envelope (reprint, if necessary, to maintain adhesive.) Print and affix the correct label(s) to the initial card/sample.

43.0 REFERENCES

- Applied Biosystems PRISM[®] 310 Instrument DNA Analyzer Diagnostic System Users Manual, 2001
- Applied Biosystems PRISM[®] 3100/3100-Avant Genetic Analyzers Users Guide, 2003
- Applied Biosystems AmpFISTR[™] Profiler Plus[®] and COfiler[®] users manual.
- Applied Biosystems - Thermo cycler manuals.
- Akane, A., Matsubara, K., Nakamura, H., Takahashi, S., and Kimura, K. Identification of the Heme Compound Copurified with DNA from Bloodstains, a Major Inhibitor of PCR Amplification, *J Forensic Sci* 1994;39:362-372.
- Budowle, B., Chakraborty, R., Carmody, G., Mason, K. Source Attribution of a Forensic DNA Profile, *Forensic Science Communications* 2000; 2:1-6.
- Budowle, B., Moretti, T.R., Baumstark, A.L., Defenbaugh, D.A., and Keys, K.M. Population Data on the Thirteen CODIS Core Short Tandem Repeat Loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans and Trinidadians, *J Forensic Sci* 1999;44:1277-1286.
- Budowle, B., Moretti, T.R., Keys, K.M., Koons, B.W., and Smerick, J.B. Validation Studies of the CTT STR Multiplex System, *J Forensic Sci* 1997;42(4):701-7.
- Budowle, B., Shea, B., Niezgoda, S., Chakraborty, R. CODIS STR Loci Data from 41 Sample Populations, *J Forensic Sci* 2001; 46:453-489.
- Budowle, B., Smerick, J.B., Keys, K.M., and Moretti, T.R. United States Population Data on the Multiplex Short Tandem Repeat Loci—HUMTHO1, TPOX and CSF1PO—and the Variable Number Tandem Repeat Locus D1S80, *J Forensic Sci* 1997;42(5):846-9. Published erratum appears in *J Forensic Sci* 1998;43(1):253.
- Chakraborty, R., Sample Size Requirements for Addressing Population Genetic Issues of Forensic Use of DNA Typing, *Human Biology* 1990;64:141-150.
- Comey, C.T., Koons, B.W., Presley, K.W., Smerick, J.B., Sobieralski, D.A., Stanley, D.M., and Baechtel, F.S. DNA Extraction Strategies for Amplified Fragment Length Polymorphism Analysis, *J Forensic Sci* 1994;39:1254-1269.
- Edwards, A., Civitello, A., Hammond, H.A., and Caskey, C.T. DNA Typing and Genetic Mapping with Trimeric and Tetrameric Tandem Repeats, *Am J Hum Genet* 1991;49:746-56.
- Fowler, J.C., Burgoyne, L.A., Scott, A.C., and Harding, H.W. Repetitive Deoxyribonucleic Acid (DNA) and Human Genome Variation—a Concise Review Relevant to Forensic Biology, *J Forensic Sci* 1988;33(5):1111-26.
- Fregeau, C.J., Bowen, K.L., and Fournay, R.M. Validation of Highly Polymorphic Fluorescent Multiplex Short Tandem Repeat Systems Using Two Generations of DNA Sequencers, *J Forensic Sci* 1999;44(1):133-66.
- GeneScan[®] Chemistry Guide, Applied Biosystems, Foster City, CA..
- GeneMapper[™] ID Software Version 3.1 User Guide, Applied Biosystems, Foster City, CA.
- GeneMapper[™] ID Software Version 3.1 and 3.2 Tutorial, Human Identification Analysis Applied Biosystems, Foster City, CA.
- Guidelines for a Quality Assurance Program for DNA Analysis, *Crime Lab Digest* 1991;18:44-75.
- Hammond, H.A., Li Jin, Zhong Y., Caskey, C.T., and Chakraborty R. Evaluation of 13 Short Tandem Repeat Loci for use in Personal Identification Applications, *Am J Hum Genet* 1994;55:175-89.
- Hochmeister, M.N., Budowle, B., Eisenberg, A., Borer, U.V., and Dirnhofer R. Using Multiplex PCR Amplification and Typing Kits for the Analysis of DNA Evidence in a Serial Killer Case, *J Forensic Sci* 1996;41(1):155-62.

LaFountain MJ, Schwartz MB, Svete PA, Walkinshaw MA, Buel E., TWGDAM validation of the AmpFISTR Profiler Plus and AmpFISTR COfiler STR multiplex systems using capillary electrophoresis. *J Forensic Sci* 2001;46:1191–8.

Lygo, J.E., Johnson, P.E., Holdaway, D.J., Woodroffe, S., Whitaker, J.P., Clayton, T.M., Kimpton, C.P., and Gill, P. The Validation of Short Tandem Repeat (STR) Loci for use in Forensic Casework, *Int J Leg Med* 1994;107:77-89.

Mansfield, E.S., Robertson, J.M., Vainer, M., Isenberg, A.R., Frazier, R.R., Ferguson, K., Chow, S., Harris, D.W., Barker, D.L., Gill, P.D., Budowle, B., and McCord, B.R. Analysis of Multiplexed Short Tandem Repeat (STR) Systems using Capillary Array Electrophoresis, *Electrophoresis* 1998;19(1):101-7.

National DNA Index System (NDIS): CODIS Standards for Acceptance of DNA Data at NDIS (1998).

National Research Council publications: DNA Technology in Forensic Science and The Evaluation of Forensic DNA Evidence. 1992 and 1996.

Quality Assurance Standards for Forensic DNA Testing Laboratories, DNA Advisory Board (1998).

Reynolds, R., Sensabaugh, G., and Blake, E. Analysis of Genetic Markers in Forensic DNA Samples Using the Polymerase Chain Reaction, *Analytical Chemistry* 1991;63:2-15.

Saiki, R.K., Walsh, P.S., Levenson, C.H., and Erlich, H.A. Genetic Analysis of Amplified DNA with Immobilized Sequence-Specific Oligonucleotide Probes, *Proc. Natl. Acad. Sci. USA* 1989;86:6230-6234.

St.Claire III RL. Capillary Electrophoresis. *Anal Chem* 1996;68(12):569R-586R.

von Beroldingen, C.H., Blake, E., Higuchi, R., Sensabaugh, G., and Erlich, H.A. Application of PCR to the Analysis of Biological Evidence. *PCR Technology Principles and Applications for DNA Amplification*, Erlich, H.A., 1989; Stockton Press, New York.

Walsh, P.S., Erlich, H.A., and Higuchi, R. Preferential PCR Amplification of Alleles: Mechanisms and Solutions. *PCR Methods and Applications* 1992;1:241-250.

Walsh, P.S., Varlaro, J., and Reynolds, R. A Rapid Chemiluminescent Method for Quantitation of Human DNA, *Nucleic Acids Research* 1992;20:5061-5065.

Waye J.S., Presley, L.A., Budowle, B., Shutler, G.G., and Fourney, R.M. A Simple and Sensitive Method for Quantifying Human Genomic DNA in Forensic Specimen Extracts, *Biotechniques* 1989;7(8):852-5.

44.0 WORKSHEETS

44.1 CODIS CASE FILE FORMS

The worksheets approved for use in CODIS Casework can be found on the WSP Portal.

45.0 REVISIONS

Introduction	version 02-2003
Calibration of Instruments	version 02-2003
Quantiblot® Hybridization and Detection (Colorimetric)	version 02-2003
Amplification of STR Loci	version 06-2003
Injection List	version 06-2003
Amplification Product Preparation	version 06-2003
Guidelines For Evaluating DNA Typing Profiles	version 06-2003
STR Case File Review	version 06-2003
Table of Contents	version 03-2004
Amplification of STR Loci-50µl	version 03-2004
Amplification of STR Loci-25µl	version 03-2004
Quantiblot® Hybridization and Detection (Colorimetric)	version 03-2004
Review of Felon/Contracted Samples	version 03-2004
QIAamp® Mini Column	version 05-2004
Extraction For Bone	version 05-2004
Table of Contents	version 05-2004
Quality Assurance/Quality Control	version 02-2005
Calibration of Instruments	version 02-2005
Guidelines for Evaluating DNA Typing Profiles	version 02-2005
Review of Felon/Contracted Samples	version 02-2005
Statistical Interpretation of STR Typing	version 04-2005
QIAamp® Micro Column	version 07-2005
Guidelines for Evaluating DNA Typing Profiles	version 09-2005
Amplification of STR Loci – 10µl	version 09-2005
CE Instrument Disassembly	version 09-2005
STR Case File Content	version 09-2005
Reagent Preparation	version 09-2005
Processing of Convicted Offender Samples	version 09-2005
Organic Non-Differential Extraction	version 09-2005
Organic Differential Extraction For Semen Stains	version 09-2005
Table of Contents	version 09-2005
3100 CE Assembly and Disassembly	version 09-2005
3100 CE Instrument Starting a Run	version 09-2005
3100 CE Instrument Shut Down	version 09-2005
Constructing a New Plate Record for the 3100	version 09-2005
Amplification Product Preparation 3100	version 09-2005
3100 Spectral Calibration	version 09-2005
Post- Installation GeneMapper® Set Up of Analytical Parameters	version 09-2005
Guidelines for Evaluating CO DNA Typing Profiles using GM ID	version 09-2005
Review of Convicted Offender Samples	version 09-2005
Amplification of STR Loci-50µl	version 09-2005
Amplification of STR Loci-25µl	version 09-2005
Amplification of STR Loci-10µl	version 09-2005
Introduction	version 09-2005
References	version 09-2005
Chelex® Extraction for Blood	deleted 09-2005
Chelex® Extraction for Semen Stains	deleted 09-2005

Chelex [®] Extraction for Hair	deleted 09-2005
Chelex [®] Extraction for Envelope Flaps & Stamps	deleted 09-2005
Introduction	version 03-2006
Table of Contents	version 03-2006
ABI 7000 Instrument; Maintenance	version 03-2006
AB 7000 Instrument; Creating Detectors	version 03-2006
AB 7000 Instrument; Creating a Plate Document Template	version 03-2006
ABI 7000 Instrument; Creating a Plate Document	version 03-2006
Quantifiler [™] –DNA Quantification Standards Preparation	version 03-2006
Quantifiler [™] – DNA Quantification Reaction Preparation	version 03-2006
Quantifiler [™] and AB 7000 Data Analysis and Interpretation	version 03-2006
Amplification of STR Loci-50µl	version 03-2006
Amplification of STR Loci-25µl	version 03-2006
Amplification of STR Loci-10µl	version 03-2006
References	version 03-2006
Table of Contents	version 04-2006
Wallac DBS Puncher: Instrument Maintenance	version 04-2006
Wallac DBS Puncher Protocol; Electronic Barcode...Tracking	version 04-2006
Wallac DBS Puncher Protocol; Manual Sample Tracking	version 04-2006
3100 CE Assembly and Disassembly	version 04-2006
Constructing a New Plate Record for the 3100	version 04-2006
Amplification Product Preparation 3100	version 04-2006
Post- Installation GeneMapper [™] Set Up of Analytical Parameters	version 04-2006
Guidelines for Evaluating CO DNA Typing Profiles using GM ID	version 04-2006
ABI 7000 Instrument; Maintenance	version 05-2006
AB 7000 Instrument; Creating a Plate Document Template	version 05-2006
Quantifiler [™] –DNA Quantification Standards Preparation	version 05-2006
Quantifiler [™] and AB 7000 Data Analysis and Interpretation	version 05-2006
Amplification of STR Loci-50µl	version 05-2006
Amplification of STR Loci-25µl	version 05-2006
QIAGEN BioRobot 8000: Introduction	version 05-2006
QIAGEN BioRobot 8000: DNA IQ [™] Sample Prep	version 05-2006
QIAGEN BioRobot 8000: DNA Extraction Setup	version 05-2006
QIAGEN BioRobot 8000: Quantifiler Setup	version 05-2006
QIAGEN BioRobot 8000: Normalization CODIS	version 05-2006
QIAGEN BioRobot 8000: Amplification Reaction Setup	version 05-2006
References	version 05-2006
Quantifiler [™] –DNA Quantification Standards Preparation	version 06-2006
Table of Contents	version 12-2006
Introduction	version 12-2006
Amplification Product Preparation 3100	version 12-2006
310 CE Instrument Set-up GeneMapper [®] ID	version 12-2006
310 Sample Sheet GeneMapper [®] ID	version 12-2006
310 Injection List - GeneMapper [®] ID	version 12-2006
310 Matrix GeneMapper [®] ID	version 12-2006
310 Sample Sheet GeneMapper [®] ID	version 12-2006
310 Size Standard GeneMapper [®] ID	version 12-2006
310 Analysis Parameters GeneMapper [®] ID	version 12-2006
Guidelines for Evaluating CO DNA Typing Profiles using GMID	version 12-2006
Guidelines for Evaluating DNA Typing Profiles	version 12-2006

Guidelines for Evaluating 310 GMID DNA Typing Profiles	version 12-2006
Review of Convicted Offender Samples	version 12-2006
Reagent Preparation	version 12-2006
Quantiblot [®] Hybridization and Detection (Colorimetric)	deleted 12-2006
Slot Blot Preparation (Biorad) for Quantiblot [®] Kit	deleted 12-2006
Slot Blot Preparation (Gibco-BRL) for Quantiblot [®] Kit	deleted 12-2006
Table of Contents	version 01-2007
3130xl Instrument Set-up and Disassembly	version 01-2007
Constructing a New Plate Record for the 3100	version 01-2007
3100/3130xl Instrument Starting a Run	version 01-2007
3100/3130xl Spectral Calibration	version 01-2007
3130xl CE Instrument Shut Down	version 01-2007
Amplification Product Preparation 3100/3130xl	version 01-2007
3130xl Cleaning and Calibration Schedule	version 01-2007
STR Case File Review	version 01-2007
Guidelines for Evaluating CO DNA Typing Profiles using GM ID	version 01-2007
Review of Convicted Offender Samples	version 01-2007
Extraction for Bone	version 01-2007
Reagent Preparation	version 01-2007
Review of Convicted Offender Samples	version 02-2007
3130xl Cleaning and Calibration Schedule	version 02-2007
Amplification Product Preparation 3100/3130/3130xl	version 02-2007
3130 Instrument Set-up and Disassembly	version 02-2007
3130 Instrument Starting a Run	version 02-2007
3130 Spectral Calibration	version 02-2007
3130 Spatial Calibration	version 02-2007
Table of Contents	version 02-2007
Introduction	version 02-2007
Table of Contents	version 04-2007
Guidelines for Evaluating DNA Typing Profiles	version 04-2007
Guidelines for Evaluating GMID DNA Typing Profiles	version 04-2007
3130 Spectral Calibration	version 04-2007
3130 Spatial Calibration	version 04-2007
3100 CE Assembly and Disassembly	version 04-2007
Quality Assurance/Quality Control	version 04-2007
Amplification of STR Loci – Identifiler [®] 25 µl	version 06-2007
Amplification of STR Loci – Identifiler [®] 12.5 µl	version 06-2007
Table of Contents	version 06-2007
Introduction	version 06-2007
3100/3130xl Spectral Calibration	version 06-2007
3100 GeneMapper Setup Analytical Parameters	version 06-2007
QIAGEN BIROBOT [®] UNIVERSAL; INTRODUCTION	version 12-2007
QIAGEN BIROBOT [®] UNIVERSAL; Preparation of Samples for DNA IQ™	version 12-2007
Extraction – CODIS	
QIAGEN BIROBOT UNIVERSAL; DNA EXTRACTION SETUP - CODIS	version 12-2007
Qiagen BioRobot UNIVERSAL; Quantifiler Setup - CODIS	version 12-2007
QIAGEN BIROBOT UNIVERSAL; Normalization of Quantified DNA - CODIS	version 12-2007
QIAGEN BIROBOT UNIVERSAL; Amplification reaction Setup – CODIS	version 12-2007
Sample Sheet	removed 12-2007

CE INSTRUMENT SET-UP	removed 12-2007
CE Instrument Disassembly	removed 12-2007
Injection List	removed 12-2007
Matrix	removed 12-2007
Size Standard File	removed 12-2007
Analysis Parameters File	removed 12-2007
Guidelines for Evaluating DNA Typing Profiles	removed 12-2007
Table of Contents	version 12-2007
Introduction	version 12-2007
QUALITY ASSURANCE/QUALITY CONTROL	version 12-2007
Calibration of Instruments	version 12-2007
Non-Differential Organic Extraction	version 12-2007
QIAamp [®] Micro Column	version 12-2007
BSA as a PCR Amplification Facilitator	version 12-2007
Centricon [®] Concentration of DNA	version 12-2007
Microcon [®] Concentration of DNA Procedure	version 12-2007
QIAamp [®] Micro Column	version 12-2007
ABI Prism [®] 7000 Instrument Maintenance	version 12-2007
Quantifiler [™] and ABI Prism [®] 7000 SDS Instrument Data Analysis and Interpretation	version 12-2007
Amplification of STR Loci – 50 µl	version 12-2007
Amplification of STR Loci – 25 µl	version 12-2007
Amplification of STR Loci – 10 µl	version 12-2007
3130 Instrument Set-Up and Disassembly	version 12-2007
3130 CE Starting a Run	version 12-2007
310 Injection List – GeneMapper [®] Id	version 12-2007
Amplification Product Preparation 310	version 12-2007
Amplification Product Preparation 3100/3130/3130XL	version 12-2007
3100/3130XL CE Spectral Calibration	version 12-2007
POST-INSTALLATION GENEMAPPER [®] Set Up of Analytical Parameters	version 12-2007
Guidelines For Evaluating Genemapper [®] ID DNA Typing Profiles	version 12-2007
Guidelines for Evaluating Convicted Offender DNA Typing Profiles Using GeneMapper [®] ID	version 12-2007
Statistical Interpretation of STR DNA Typing	version 12-2007
Reagent Preparation	version 12-2007
STR CASE FILE CONTENT	version 12-2007
STR CASE FILE REVIEW	version 12-2007
Separation of CODIS and Casework Procedures Manuals	version 09-2008
Constructing a New Plate Record for the 3130XL	version 01-2009
Microcon [®] Concentration of DNA Procedure	version 04-2009
Reagent Preparation	version 04-2009