



**TENNESSEE BUREAU OF INVESTIGATION**  
*Forensic Services Division*

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CODIS Standard Operating Procedures Manual  
CODIS Databasing Manual

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Tennessee Bureau of Investigation

# **CODIS Databasing Manual**



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## **1.0. Introduction**

### **Introduction for CODIS Databasing Test**

Short tandem repeat (STR) markers are polymorphic DNA loci that contain a repeated nucleotide sequence. The STR repeat unit ranges from 2-7 nucleotides in length. A majority of the STRs that have been evaluated by the forensic community are composed of four nucleotide repeat units. The number of repeat units within a STR locus may differ, so alleles of many different lengths are possible (polymorphic). Therefore, STR loci are useful for human identification purposes.

STR loci can be amplified using the polymerase chain reaction (PCR) process and the PCR products are then analyzed by electrophoresis to separate the alleles according to size. PCR-amplified STR alleles can be detected using various methods, such as fluorescent dye labeling, silver staining or fluorescent dye staining.

In capillary electrophoresis (CE), dye-labeled amplified fragments are separated by size as they migrate through a sieving medium contained within a narrow (50  $\mu$ m) diameter capillary. The use of a narrow diameter capillary enables efficient heat dissipation during electrophoresis and allows much higher separation voltages to be used.

As the labeled amplified fragments migrate through the capillary and pass a detection window, they are illuminated by a laser. The dyes attached to the fragments are excited and emit light at a specific wavelength for each dye. This data can be then collected, analyzed and translated by the appropriate software.

This manual covers appropriate laboratory procedures for the preparation of EasiCollect+ FTA cards, punching systems, direct amplification, typing of human DNA for autosomal STR profiles using the GlobalFiler® amplification kit, and capillary electrophoresis of databasing samples.

**For accessioning, sample inventory, and long-term sample storage and retention**, see section 7.0 (Offender), 9.0 (Missing Persons and Relatives of Missing Persons), and 10.0 (Arrestee) of the CODIS Unit Manual. Accessioning and initial sample inventory and storage of EasiCollect+ kits will typically occur in the Jackson Laboratory while final inventory and storage, as well as buccal swab kits will typically occur at the Nashville Laboratory. For Missing Persons and Relatives of Missing Persons that are non-evidentiary samples processed by the CODIS Unit at the Jackson Laboratory, these samples will follow the appropriate workflow depending on the type of sample substrate received.

**Initial sample storage:** samples received by the CODIS Unit in Jackson will be stored in an area with limited access upon receipt until transfer to the Nashville Laboratory for final retention and/or destruction. Any expungement notifications received prior to sample processing should be removed from the accessioning process and sent to the Nashville Laboratory for destruction. Transfer to the Nashville Laboratory can be done through the mail (with tracking number) or in person by a TBI employee. Packing list/inventory list for shipments, list email/share drive to CODIS unit in Nashville and included in pkg. checked prior to shipment. (barcode shipment lists/pkgs include db# and plate#s).



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**For Upload and sample/match management, and verification**, see section 7.0 (Offender), 10.0 (Arrestee), and 12.0 (Match Management for TBI Samples) of the CODIS Unit Manual, this will typically occur in the Nashville Laboratory.

**For expungements**, see section 13.0 (Expungement – Convicted Offenders), 14.0 (Expungement – Arrestee), and 15.0 (Expungement – Sex Offender) in the CODIS Unit Manual, this will typically occur in the Nashville Laboratory.



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## **2.0. Reagents and Supplies**

The following general instructions are applicable in the preparation of all reagents:

- Clean disposable gloves, a mask and lab coat should be worn at all times while preparing reagents.
- Use graduated cylinders or pipettes closest in capacity to the volume being measured for preparing liquid reagents.
- Prepare all reagents in an area free of any source of DNA, including extracted DNA and amplified DNA. Reagents prepared in the Forensic Biology Reagent Preparation Room should be transported to the CODIS lab at a minimum in a closed container and wearing gloves.
- Label all reagents with name of reagent, date prepared, expiration date and preparer's initials. If applicable, label reagent with storage conditions and sterilization date.
- Complete Reagent Quality Control Sheet for each reagent prepared.
- For a commercially purchased reagent not having an expiration date, the expiration date will be five years from the date of receipt unless noted otherwise in policy.
- In-house prepared reagents will be discarded after one year unless noted otherwise in policy.

Any specific temperature mentioned in this protocol refers to the temperature at which the equipment is set given its temperature range, determined by the manufacturer and monitored by the Forensic Biology and/or CODIS Unit's staff.

Some examples are: 5°C is considered to be refrigerated. A refrigerator set for 5°C could read between 2°C and 8°C depending on the cyclic range during the time the temperature was taken. A freezer set for -20°C could read between -15°C and -25°C depending on its range.

NOTE: All consumable products provided by Life Technologies are guaranteed to perform as specified for one year from the date received, unless otherwise indicated on the product label.

**FOR A LIST OF CRITICAL REAGENTS SEE APPENDIX A.**

**FOR ANY FORENSIC BIOLOGY REAGENTS AND SUPPLIES USED, SEE THE FORENSIC BIOLOGY STR MANUAL.**

## **2.1 Reagents and Supplies for Punching Systems and Direct STR Amplification**

Brand name and part number after reagent / supply items is the recommended item, not necessarily the brand in use. Aliquot storage volumes are recommended and may be adjusted.



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#### **96-WELL OPTICAL REACTION PLATE:**

P/N 4326659 (bulk)

#### **GLOBALFILER® AMPLIFICATION KIT (200 reactions or 1,000 reactions):**

Life Technologies (P/N 4476135 or 4482815)

- GlobalFiler® Master Mix contains: enzyme, salts, dNTPs, bovine albumin, enzyme and sodium azide in buffer and salt.
- GlobalFiler® Primer Set contains: Forward and reverse primers to amplify human DNA targets.
- *GlobalFiler® control DNA 007 contains: 0.10 ng/μg human male genomic DNA from cell line in 0.05% sodium azide and buffer. (NOT USED), see DNA CONTROL 007 (2ng/μL)*
- GlobalFiler® Allelic Ladder contains amplified alleles.

Reagents should be stored at -15°C to -25°C upon receipt and then stored at 2°C to 8°C after initial use. The reagents should be protected from light.

#### **DNA CONTROL 007 (2ng/μL)**

Life Technologies (P/N A45874)

#### **MICROAMP STRIP COVERS:**

MicroAmp 8-Cap Strip

Life Technologies (P/N N801-0535)

#### **PIPETTE TIPS:**

Aerosol Resistant Pipette Tips (or equivalent)

10 μL – Fisher, 21-402-482

20 μL – Fisher, 21-236-4

100 μL – Fisher, 21-236-8

200 μL – Fisher, 21-236-1

1000 μL – Fisher, 21-236-2A



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BrandTech 0.5 mL Tips for Repeat Pipetter:

Brand Tech Scientific, P/N 702684

50µl Conductive Filter Tips, Hamilton (P/N 235948)

SCREW CAP MICRO TUBES, STERILE (2.0 mL):

Tube: ThermoFisher (P/N 342810-0020) or similar.

Cap: ThermoFisher (P/N 342820-0110) or similar.

STERILE WATER:

In-house Reverse Osmosis Water (R.O.) or In-house deionized or sterile (Fisher Scientific BP24701, Life Technologies AM9930).

TE BUFFER:

See Forensic Biology STR Manual

## **2.2 Reagents and Supplies for Capillary Electrophoresis**

24-CAPILLARY ARRAY, 36cm

Life Technologies (P/N 4404687)

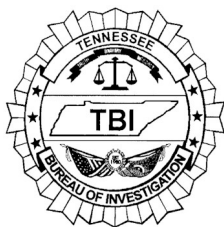
96-WELL OPTICAL REACTION PLATE:

See Section 2.1

96-WELL STANDARD RETAINER AND BASE SET 3500/3500xL:

Life Technologies (P/N 4410228)

ANODE BUFFER CONTAINER



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Life Technologies (P/N 4393927)

#### **CATHODE BUFFER CONTAINER**

Life Technologies (P/N 4408256)

#### **CONDITIONING REAGENT**

Life Technologies (P/N 4393718)

#### **DS-36 Matrix Standard (J6 Dye Set)**

Life Technologies (P/N 4425042)

#### **FORMAMIDE, DEIONIZED**

HiDi Formamide pure grade

Life Technologies (P/N 4311320)

Allow bottle to thaw, aliquot up to 1000  $\mu$ L in each tube and store for up to 3 months at  $-15^{\circ}\text{C}$  to  $-25^{\circ}\text{C}$ . Use only the needed amount of an aliquot per set of samples and discard any unused portion(s) of aliquoted deionized formamide.

**Warning! Chemical Hazard.** Formamide is a known teratogen and is harmful by inhalation, skin contact or ingestion. It can cause birth defect. Use in a well-ventilated area; use chemical-resistant gloves and safety glasses when handling. Wash thoroughly after handling formamide.

#### **GENESCAN 600 LIZ® SIZE STANDARD VERSION 2.0**

Life Technologies (P/N 4408399)

GeneScan™ 600 LIZ® Size Standard v.2.0 provides 36 single stranded labeled fragments of: 20, 40, 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, 460, 480, 500, 514, 520, 540, 560, 580 and 600bp.

**PERFORMANCE OPTIMIZED POLYMER (POP-4) (96 samples, 384 samples, or 960 samples):**



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Life Technologies (P/N A26070, 4393715, or 4393710)

#### **PIPETTE TIPS:**

See Section 2.1

#### **SCREW CAP MICRO TUBES, STERILE (2.0 mL):**

See Section 2.1

Or NS RNase-free microfuge tube (1.5mL), Life Technologies (P/N AM12450)

#### **SEPTA, 96-WELL PLATE:**

Life Technologies (P/N 4412614)

#### **SEPTA, CATHODE BUFFER CONTAINER:**

Life Technologies (P/N 4410715)

#### **STERILE WATER**

See Section 2.1



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### **3.0. Laboratory Setup**

The sensitivity of PCR amplification kits necessitates precautions to avoid contamination of samples yet to be amplified from other sources of DNA. To minimize the potential for laboratory-induced DNA contamination, time and/or space will separate kit accessioning, punching/PCR setup, and DNA typing/amplified PCR products. The following section addresses the dedicated equipment and supplies, special precautions to be taken, and controls to be used. In the event of cross-contamination of a sample from another DNA source during any stage of the DNA typing process, the technical leader will be notified, and documentation must be placed in the affected databasing plate packet(s).

#### **3.1 Accessioning Room**

This work area should be used for the opening, labeling, and documenting of databasing kits with dedicated equipment and supplies. Disposable gloves should be worn at all times when handling kits.

#### **3.2 Card Preparation Area**

This work area should be used for the preparation of cards for punching. This dedicated area of the pre-amplification room and the supplies in this area should be cleaned before and after use. Supplies may also be UV treated.

#### **Special Precautions:**

1. Lab coats, gloves, and mask should be worn at all times when handling cards.
2. Each month and as needed, clean all work surfaces thoroughly with 10% dilution of household bleach or 70% ethanol (or isopropyl alcohol). Use disposable bench paper or absorbent pads to prevent the potential accumulation of human DNA on permanent work surfaces. Use caution when handling bleach.

#### **3.3 Punching and PCR Setup Work Area**

This work area is used for punching instruments and the preparation and combining PCR reagents in PCR reaction plates. This dedicated area of the pre-amplification room and the supplies in this area should be cleaned before and after use. Supplies may also be UV treated.

#### **Special Precautions:**



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1. Lab coats, gloves, and mask should be worn at all times when handling cards and reagents.
2. Use sterile disposable barrier pipette tips and microcentrifuge tubes or reagent containers.
3. Avoid touching the inside surface of the tube caps.
4. Always change pipette tips between each sample containing DNA.
5. Record the lot(s) of reagents used in each set of samples so that if contamination occurs it can be traced more readily.
6. Include controls with each plate to check for the presence of contaminating DNA in reagents and proper function of the reagent/method.
7. Each month and as needed, clean all work surfaces thoroughly with 10% dilution of household bleach or reagent alcohol. Use disposable bench paper to prevent the potential accumulation of human DNA on permanent work surfaces. Use caution when handling bleach.
8. Do not use kit components beyond the expiration date.
9. Store the DNA amplification reagents together in the rack or the box provided, which will serve as a barrier against possible contamination by exogenous DNA. The rack or box is stored in a refrigerator that does not contain evidence samples.
10. If a microamp tube rack used to transport tubes to the post amp room touches any surface in the amplified DNA work area, it must be decontaminated by washing and/or irradiating with UV light before return to the amplification setup area.

#### **3.4 Amplified DNA Work Area (Post-Amplification Room)**

This work area shall be a physically separate area and used only for those activities that involve the handling of amplified DNA. This includes thermal cyclers used to produce amplified DNA, capillary electrophoresis of amplified DNA, handling of the allelic ladders, storage of frozen amplified DNA, and waste disposal of amplified DNA product and solutions.

Dedicated Equipment and Supplies: amplified DNA or equipment and supplies used to handle amplified DNA should not be taken out of the amplified DNA work area. Items that must be removed from the amplified DNA work area/post-amplification room (e.g. pipettes for calibration) must be decontaminated with bleach, alcohol, or UV light exposure.

#### **Special Precautions:**

1. Everyone working with amplified product is required to wear a disposable lab coat and gloves. If entering the room for other reasons (e.g. to check on a run), only gloves need to be worn.
2. Always remove gloves when leaving the amplified DNA work area to avoid the transfer of amplified DNA into other work areas.



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3. Change gloves when they may have come in contact with amplified DNA.
4. Use disposable bench paper or absorbent pads to cover the work area used to perform the typing steps to prevent the potential accumulation of amplified DNA on permanent work surfaces. Diluted bleach or alcohol should be used as needed to wash exposed work surfaces. Soap and water can also be used to clean work surfaces.
5. Store reagents as small aliquots to minimize the number of times a given tube of reagent is thawed.
6. Record the lot(s) of reagents used in each set of samples so that if contamination occurs it can be traced more readily.
7. Use the thermal cycler only for amplification and denaturation of DNA for typing.
8. Store plates of amplified DNA in the freezer at -20°C. Do not store amplified DNA samples in the same area as reagents.
9. Store the allelic ladders frozen until use.
10. The post-amplification room is designed to have a one-way flow of people leaving the room. A person desiring to exit the post amp room can only exit into the de-gowning vestibule or hallway.
11. Anyone exiting the post-amp room should wash his/her hands in the de-gowning vestibule sink.
12. Gloves will be placed in a trash container and lab coats may be stored in the gowning or de-gowning vestibule for re-use. All coats should be discarded when soiled or as needed.
13. All trash in the post amp room will exit through the de-gowning vestibule door leading into the hallway. No trash should exit through the door leading into the laboratory.
14. Doors to the post-amplification room will remain closed at all times except for passage to and from the room.



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## **4.0. Guidelines for Control Samples**

It is essential that proper control samples are included when samples are processed. The typing results obtained from these controls are important for the interpretation of the typing results obtained from samples. Controls are described below.

### **4.1 Amplification Controls**

**Amplification Blank (Negative Control):** An amplification blank must be used with each set of amplifications. This sample contains all of the amplification reagents with no DNA template. For this control, 15 µL of TE must be added for the GlobalFiler® amplification. It is recommended that two negative controls be included on the plate.

**Positive Control:** With each set of amplifications, a sample containing DNA of known types must be amplified and carried through the remainder of the typing procedure. The DNA Control 007 is included in the GlobalFiler® Amplification Kits will not be used, a 2ng/µL DNA Control 007 will be purchased separately or used from the Yfiler Plus® Amplification Kit. If the positive control from the Yfiler Plus® kit is used, it must go through the quality control check with the GlobalFiler® kit at 27 cycles. It is recommended that 2µL of the 2ng/µL DNA Control 007 be used for a 27-cycle amplification and that at least one positive control be included on the plate.

#### **Documenting Controls:**

The positive and negative controls will be documented on the punch and direct amplification plate worksheet along with the samples being amplified. The controls will be identified with the date of amplification, a sequential number, and the scientist's initials, e.g. "POS080624-1BRE, POS080624-2BRE, POS080624-3BRE, etc." and "NEG080624-1BRE, NEG080624-2BRE, NEG080624-3BRE, etc."

### **4.2 Quality Control Samples**

At least four samples from a previously punched plate should be processed each month. The concordance of these samples should be documented and a review of the data shall be performed by a technical reviewer.



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Concordance is the comparison of alleles obtained for consistencies or discrepancies to determine if the profiles are from the same sample or not. A profile with a discordant allele, which cannot be explained by dropout or the presence of an artifact, or a profile does not have sufficient data to determine concordance, should be re-processed or additional testing should be performed.



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## **5.0. Punching**

Punching systems used with databasing samples take uniform sample sizes from FTA cards. FTA cards denature proteins that can degrade DNA and lyses cells while protecting DNA and preserving the sample. Samples can then be consistently punched in preparation for direct amplification as well as being used for traditional extraction and storage. The design of punching systems also incorporates cleaning and contamination prevention features such as cleaning punches to prevent contamination by the punch head, humidity and ionization conditions to control static and punch placement, and vacuum system for dust removal.

### **5.1 Hamilton easyPunch**

The Hamilton easyPunch is an automated robotic punching and liquid handling system on a Hamilton STARlet platform for direct PCR amplification from FTA cards into a 96-well PCR plate.

Punch size: 1.2mm

Software: Hamilton Method Manager 2v1.0, Hamilton Run Control v4.5.0.7977, and easyPunchImaging v1.1.0

Procedure:

#### Preparation:

1. Turn on the instrument.
2. Pull carriers to loading deck and close front cover.
3. Perform the daily or weekly maintenance (first use of day/week; weekly includes the daily maintenance).
4. Check sample order against punch and direct amplification plate worksheet.
5. Load up to 20 sample cards into each magazine (DO NOT leave empty slots between cards), up to 88 cards and 5 magazines can be used. Sample cards are loaded with the *Sampling area* facing left and the *Cleaning area* toward the top; cards loaded from left to right in the magazine.



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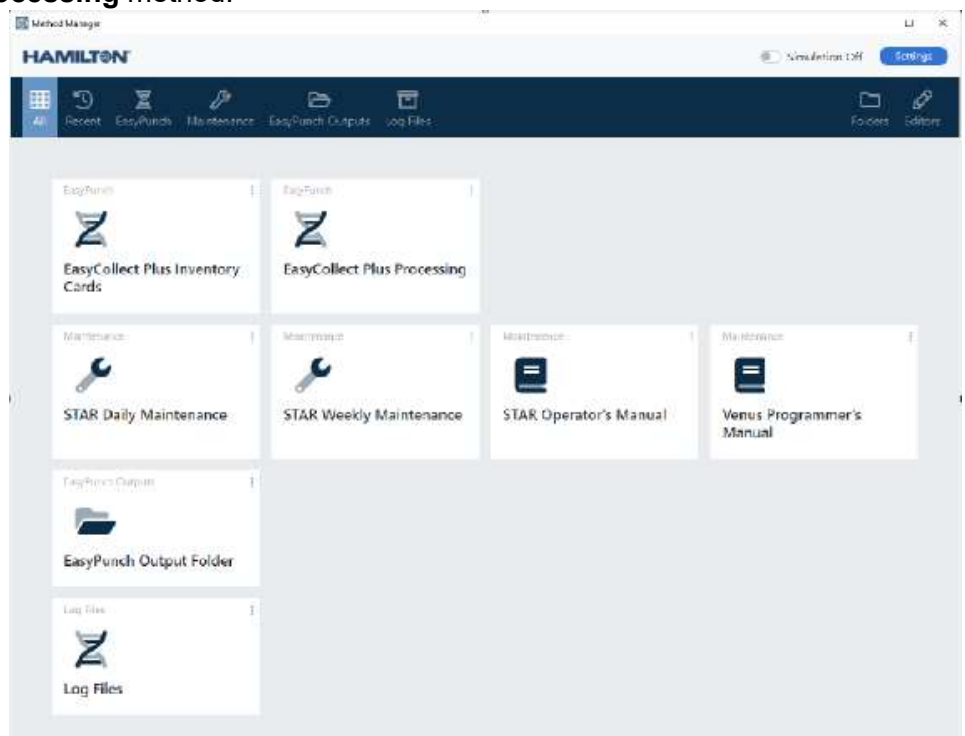
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6. In the Hamilton Method Manager, select the **EasyCollect Plus Processing** method.



7. Select **Start method using input dialogs with default settings** or **Start method using a worklist**. OK. Follow the prompts in the subsequent windows.



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**HAMILTON**  
Method Settings

Please select a setting.

Mode:

- ☒ Start method using input dialogs with default settings
- ☐ Start method using input dialogs with last used settings
- ☐ Start method using a worklist

Press OK to continue.

Ok Cancel

8. Input the *Number of cards*, *Number of magazines*, *Number of cleaning punches per card* (3 Cleaning punches), *Volume of positive controls* (2 Microliter), *Positions of positive controls per plate* (e.g. B1 and second to last well), *Positions of negative controls per plate* (e.g. A1 and third to last well), *Positions of empty wells per plate* (used for allelic ladders; e.g C1 and last well or one per CE injection), and no changes should be needed for the remaining settings. OK.

**HAMILTON**  
Method Settings

Please enter the settings for the method.

| Parameter                                           | Value | Range/Description         |
|-----------------------------------------------------|-------|---------------------------|
| Number of cards                                     | 5     | Cards                     |
| Number of magazines                                 | 1     | Magazines                 |
| Number of punches per card and plate                | 1     | Punches                   |
| Number of punches per card in one well              | 1     | Punches                   |
| Number of cleaning punches per card                 | 3     | Cleaning punches          |
| Maximum retries if no punch is found in target well | 1     | Retries                   |
| Number of cleaning punches if no punch is found     | 0     | Cleaning punches          |
| Punch diameter                                      | 1.2   | Millimeter                |
| Volume of pcr amplification mix                     | 10    | Microliter                |
| Volume of positive controls                         | 2     | Microliter                |
| Positions of positive controls per plate            | B1    | A1-H12 (separated by ";") |
| Positions of negative controls per plate            | A1    | A1-H12 (separated by ";") |
| Positions of empty wells per plate                  | C1    | A1-H12 (separated by ";") |

Press OK to continue.

Ok Cancel



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9. On the next screen, if using separate cards for cleaning punch, change selection to Yes. If not using separate card, the setting should remain No. No other changes should be needed for the remaining settings. OK.

**HAMILTON**  
Method Settings

Please select a setting.

☒ Use separate card for cleaning punches  
☐ No  
☒ Yes

☒ Use sample validation  
☒ No  
☐ Yes

☒ Read card barcodes  
☒ No  
☐ Yes

☒ Punch strategy  
☐ Center of sampling area  
☒ Center of sample  
☐ Top to down  
☐ Down to top  
☐ Left to right  
☐ Right to left  
☐ Center of biggest inner circle

☒ Create two plates with same samples  
☒ No  
☐ Yes

Press OK to continue.

Ok Cancel

**HAMILTON**  
Method Settings

Please select a setting.

☒ Layout for punches at plate :  
☐ Rowwise  
☐ Columnwise  
☐ Randomized

Press OK to continue.

Ok Cancel

10. Load the magazines following the method instructions (only Magazine 1-5 will be used for sample cards, load Magazine 1 first; Magazine 10 will be used if Cleaning Cards are used). Ensure the front cover is closed. CONTINUE.



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11. Vortex and spin the GlobalFiler® Master Mix and Primer Set tubes briefly. Prepare the GlobalFiler® master mix as indicated in the module in a 2.0mL screw cap microcentrifuge tube. In a separate 2.0mL screw cap microcentrifuge tube, add the amount of TE Buffer indicated in the module.

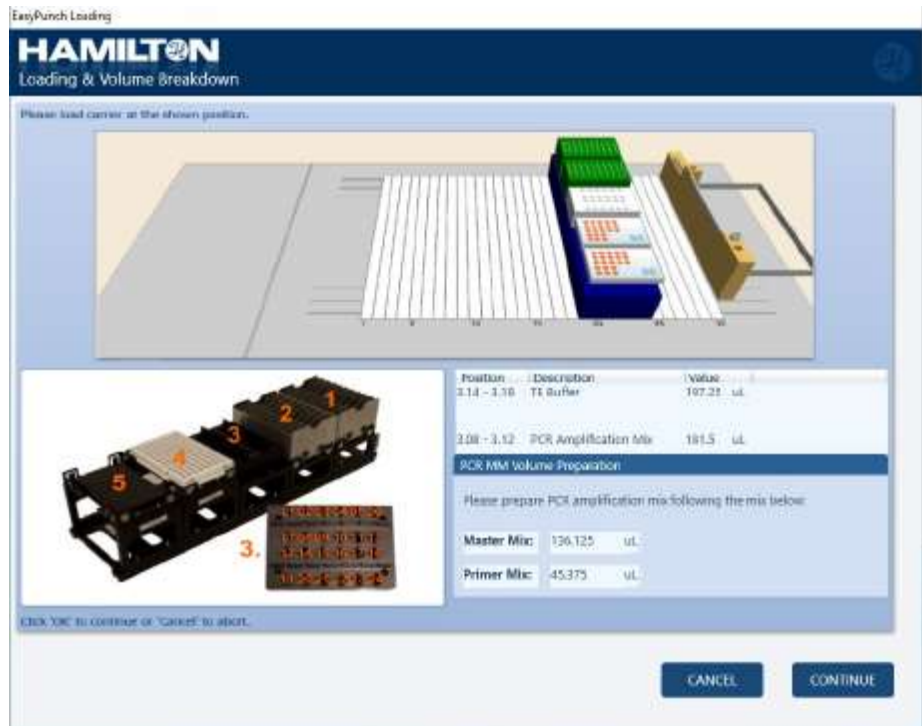


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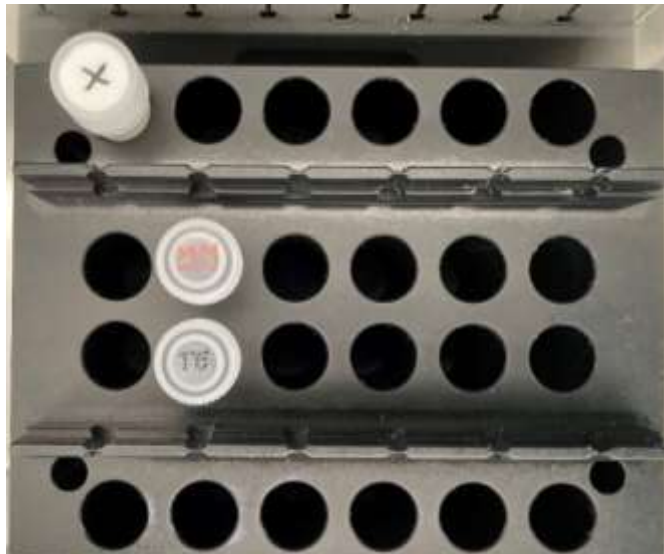
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12. Vortex the Control DNA 007 tube (2ng/μL). Spin the tube briefly in a microcentrifuge to remove any liquid from the cap.
13. Load the TE buffer, GlobalFiler® master mix, and DNA 007 control to the reagent tube rack positions listed in the method.



14. Load a 96-well reaction plate to the plate holder (Output Plate 1), ensure plate is fully seated. Ok.



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15. If prompted, reload 50 $\mu$ L filtered tips (prompted when there are less than 10 tips). Ok.



16. Ensure that the waste troughs are on the tray. Ok. The run will begin.



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#### EasyPunch robotic method (instrument process):

1. Dispenses TE buffer, amplification master mix, and positive control into necessary wells.
2. Read card, performs three cleaning punches into waste troughs, then punches a sample from the card into the designated well of the plate.
3. Images are captured of the plate before punching, of the card before cleaning punches, of the card before sample punching, and of the plate after punching.

#### Post-run:

1. Ensure the run has completed properly and check the plate wells.
2. From Method Manager, select the OutputFiles icon and save a copy of the OutputFile to the external hard drive in a folder titled with the plate#.
3. If there is a quality issue during the run, view the plate and/or card images under C:\Program Files (x86)\Hamilton\easyPunch Imaging Application\database\pictures and save a copy of the pictures to the external hard drive in the plate# folder..
4. Place a MicroAmp 8-Cap Strip on each column of the plate used.
5. Remove and store sample cards/magazines.
6. Wipe the punching deck and magazine carrier with 70% isopropanol.
7. Empty waste troughs (if needed).
8. Complete the instrument log, close all software, and turn off the instrument.



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9. Carry the plate into the Post-Amplification room, centrifuge at 3000 rpm for about 20 seconds, place on the GeneAmp® PCR System 9700 thermal cycler, and close the cover.
10. Turn on thermal cycler (if not already on), select the **gf-27c** program to run, and press Start twice.
11. To ensure that no problems were experienced during the run, check the run or the history after the completed program is stopped.
12. After amplification, remove plate from thermal cycler and proceed to capillary electrophoresis or store the amplified products in a freezer at -20 C. Store amplified DNA samples separate from all PCR amplification reagents. Amplified DNA may be discarded after the analysis of the plate is technically reviewed.

## **5.2 BSD600 Ascent A2**

The BSD600 Ascent A2 is a semi-automated punching system for direct amplification from FTA cards into a 96-well PCR plate.

Punch sizes: 1.2mm and 1.7mm

Software: BSD Studio v1.7.4.0 and BSD Integrator v1.4.3.0

Procedure:

#### Pre-run maintenance:

- It is recommended that the pre-run maintenance be performed approximately 30 minutes before a run is started depending on the humidity of the laboratory.
1. With the instrument off, clean the chute and punch mechanisms (see 5.7-5.8 page 19 and 15.2 pages 91-92 in the BSD manual for further instructions).
    - a. Remove punch guard and punch head, clean with alcohol, dry, blow out the punch head with canned air, and replace the punch head and punch guard.



Figure 9 - Removing the punch head

- b. Blow out the plate chamber and deck with canned air.



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- c. Remove chute by carefully disconnecting the plug (do not pull on cables) (2), unscrew the inner chute by turning it counter-clockwise (3), then push down the chute lifting lever arm and carefully remove the chute (caution inner chute slides out of outer chute easily, do not drop either piece) (4). For the inner chute, clean with alcohol, dry, and blow out with canned air. For the outer chute, clean with dry brush only and blow out with canned air.



Removing the chutes



- d. Check the water level in the humidifier jar. If the water level is below the bottom red band (jar may be raised for better viewing), unscrewing the silver filler cap and add sterile distilled water until the level is slightly above this band (do not fill above the top red band). Replace filler cap, lower jar, and ensure the outside of the jar is dry.



Figure 11 - Humidifier System



(new sponge-less humidifier bottle)

2. Turn on the instrument and computer.

**Caution:** do not turn on the instrument unless all components are dry.



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3. Open BSD Studio software (User: Admin, pwd: admin).
4. Select Start on the software start up screen.
5. Ensure that the instrument, barcode, and camera are online.
6. In settings (  gear icon in lower right corner), turn on the humidifier.



#### Preparation:

1. Check the sample order against punch and direct amplification plate worksheet and load up to 20 sample cards into the card holders.
2. Prepare the GlobalFiler® master mix. See Section 6.2 Direct PCR Amplification using GlobalFiler® with the BSD600 Ascent A2.
3. Select Test Editor.
  - a. Add
    - i. Name the test
    - ii. Type: **96 Well PCR**
    - iii. Choose any of the fill pattern options
  - b. Select test plate created
    - i. Plate info
      1. Add barcode, if used.
    - ii. Punch settings: 1.2mm is the default but can be change to 1.7mm if the majority of the plate will be the larger punch
    - iii. Fill order: may be changed to users preference, select reorder to apply
    - iv. Import file: select import setting if used, then select import file
    - v. Select well
      1. Change type to sample, liquid control, or unused
        - a. Unused is used for empty wells and ladders
      2. Fill in the well label and sample label
      3. For samples, the default punch size will be selected, but can be changed between the 1.2mm and 1.7mm.
      4. Repeat for all wells.
  - c. Exit
4. In settings (gear icon in lower right corner), turn on the ionizer.
5. Turn on the dust extraction system (external to the instrument on the left).
6. Select Punch to open the run protocols.
7. Select the plate location on the deck (1 or 2) and assign the plate, then select Close.



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8. Check the cleaning settings, click Edit to view (cards between cleaning punches 1 and number cleaning punches 3).

#### Punch protocol:

1. Select the START PUNCH RUN button and follow the instrument instructions to load the plate, select Continue.
2. Perform a cleaning punch with each punch head that will be used during the run.
3. Scan sample barcode or manually enter the sample barcode/name.
4. Slide the sample card under the card clamps. The laser dot(s) will show the location of the punch.
5. Punch the card by using either the foot pedal, selecting Punch in the software, or with the auto-trigger.
6. Check the Well Camera photo to ensure that a punch is detected in the well (when detected by camera there will be a green dot in bottom right corner of the image).

Note: If a punch is not present in the well, the punch should be located and removed before continuing with the remainder of the plate.

7. At least three cleaning punches should be performed between each sample.
8. Repeat steps 5-8 for each sample until the plate punches are complete.

Note: See BSD User Manual for 13.15 Skip Sample or 13.16 Punch Error for further instructions if needed.

9. Perform a cleaning punch with each punch head that was used during the run.
10. When punching is complete, select Show Plate Images and Platemap to ensure that each well is correct, then select End Punch Run.
11. Save the output file and images from the run to the external hard drive in a folder titled with the plate#.
12. Turn off the external dust extraction system, humidifier, and ionizer.

#### Post-run:

1. Remove the plate from the instrument and check the wells.
2. Place a MicroAmp 8-Cap Strip on each column of the plate used.
3. Empty cleaning strike waste container.
4. Wipe down the card platform with alcohol, dry, and blow out with canned air.
5. Store sample cards.
6. Complete the instrument log, close all software, and turn off the instrument.



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7. Carry the plate into the Post-Amplification room, centrifuge at 3000 rpm for about 20 seconds, place on the GeneAmp® PCR System 9700 thermal cycler, and close the cover.
8. Turn on thermal cycler (if not already on), select the **gf-27c** program to run, and press Start twice.
9. To ensure that no problems were experienced during the run, check the run or the history after the completed program is stopped.
10. After amplification, remove plate from thermal cycler and proceed to capillary electrophoresis or store the amplified products in a freezer at -20 C. Store amplified DNA samples separate from all PCR amplification reagents. Amplified DNA may be discarded after the analysis of the plate is technically reviewed.

#### **5.3 Manual Punch**

The UniCore punch is a manual punching tool for cutting sample discs from FTA cards into a 96-well PCR plate.

Punch sizes: 1.2mm and 2.0mm

Software: None

Procedure:

##### Preparation:

1. Check the sample order against punch and direct amplification plate worksheet.
2. Prepare a 96-well plate or 0.2µL amplification tubes with GlobalFiler® master mix, TE Buffer, and controls. See Section 6.2 Direct PCR Amplification using GlobalFiler® with the BSD600 Ascent A2.

##### Direct PCR Amplification using GlobalFiler® from Section 6.2:

1. Determine the number of samples to be amplified, including positive and negative controls. The Positive Control DNA (DNA 007 (2ng/µL) control, purchased separately) must be amplified with every run. The Negative Control consisting of 15µL of TE Buffer must also be included in every run.
2. Vortex the Master Mix and Primer Set. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.
3. Prepare the appropriate volume of each component needed by combining the following volumes of reagents for each reaction in a microcentrifuge tube:
  - GlobalFiler® Master Mix: 7.5µL
  - GlobalFiler® Primer Set: 2.5µL



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**Note:** Include at least 3 additional reactions in your calculation to provide excess volume for the loss that occurs during reagent transfer. See Appendix G (GLOBALFILER AMPLIFICATION MASTER MIX) in the Forensic Biology STR Typing Manual.

4. Mix thoroughly by vortexing.
5. Spin the tube briefly in a microcentrifuge to remove any liquid from the cap.
6. Dispense 10 $\mu$ L of the reaction mix into each well of the 96-well plate that will contain a negative control, positive control, or sample.
7. Add 15 $\mu$ L of TE Buffer into each well of the 96-well plate that will contain a negative control or sample. Add 13 $\mu$ L of TE Buffer to well(s) that will contain a positive control.
8. Vortex the Control DNA 007 tube (2ng/ $\mu$ L). Spin the tube briefly in a microcentrifuge to remove any liquid from the cap. Add 2 $\mu$ L of the control DNA into each well of the 96-well plate that is a positive control.
9. The final volume in each well is 25 $\mu$ L.

#### Punch protocol:

1. Position a blank FTA card on the cutting mat and remove the cover cap from the punch tip.
2. Use the free hand to hold the card and mat steady. Firmly grip the punch and position the tip at a right angle to the card.
3. Push the tip downwards into the card with a semi-circular rotation (gentle twisting) until the tip passes through the card and contacts the cutting mat.

**Note:** Do not depress the plunger when coring sample. Do not push the tip

4. Lift the punch away from the card with the cored sample stored in the cutting tip.

**Note:** After coring, the sample is temporarily stored in the hollow cutting tip ready for preferred ejection. The punch can be rested on its side without loss of sample.

5. To eject the sample, position the tip over the waste container and depress the plunger. Remove the tip slowly to ensure the sample is fully deposited.
6. Repeat steps 1-5 two additional times.
7. Position a sample card on the cutting mat and repeat steps 2-5, ejecting the sample into the correct well of the plate.
8. Wipe the cutting mat with a cleaning wipe, then repeat steps 1-5 three times (cleaning punches) before punching another sample.
9. When punching is complete, place a MicroAmp 8-Cap Strip on each column of the plate (if a plate is used).



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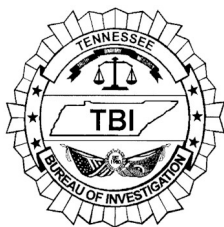
---

10. Empty the cleaning punch waste container.

#### Cleaning:

1. Aliquot alcohol into a container.
2. Dip the punch tip into the alcohol and swirl for 3-5 seconds.
3. Remove the punch tip from the alcohol and allow to air-dry for 3-5 seconds.
4. Repeat steps 2 and 3.
5. Wipe the punch tip to remove excess alcohol and wipe the outer surface of the puncher with a cleaning wipe or with bleach (bleach should be followed by ethanol and water to prevent rusting of the tip).
6. Allow the punch to dry for at least 10 minutes before next use.
7. Empty the cleaning punch container.
8. Wipe down

**Note:** Bleach (10% bleach solution), ethanol (95%)/isopropyl alcohol (70%)/reagent alcohol, and alcohol wipes can be used for cleaning.



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## **6.0. Direct Amplification**

Direct PCR amplification is a method that amplifies DNA from samples without the initial steps of extracting, purifying, or quantifying the DNA. This method can be used to amplify DNA from a variety of sources including, but not limited to, blood and saliva. Direct amplification is routinely used in high-throughput laboratories for efficient processing, small sample consumption, limited sample loss, and fewer resources and instrumentation needed. Inhibitors can affect the direct amplification process as no purification step is performed; however, databasing buccal samples are not often collected from sources containing high levels of PCR inhibitors and the commercial amplification kit buffer along with the TE buffer can typically overcome and dilute many of the inhibitors that may be encountered.

GlobalFiler® Direct, or GlobalFiler Databasing, is not a specialized kit, but terms used to distinguish a difference from the traditional GlobalFiler amplification method used for TBI casework in the Forensic Biology Unit and the direct amplification method used for databasing samples punched into an amplification plate in the CODIS Databasing Unit.

### **6.1 Direct PCR Amplification using GlobalFiler® with the Hamilton easyPunch**

1. Vortex the Master Mix and Primer Set. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.
2. Prepare the volume of GloabalFiler® master mix for at least the amounts indicated by the Hamilton easyPunch software in a 2.0mL screw cap tube.

**Note:** See Appendix G (*GLOBALFILER AMPLIFICATION MASTER MIX*) in the *Forensic Biology STR Typing Manual*.

3. Mix thoroughly by vortexing.
4. Spin the tube briefly in a microcentrifuge to remove any liquid from the cap.
5. Load the tube onto the appropriate position of the tube rack on the Hamilton easyPunch.
6. See Hamilton easyPunch protocol (preparation step 12) for next step.

### **6.2 Direct PCR Amplification using GlobalFiler® with the BSD600 Ascent A2**

1. Determine the number of samples to be amplified, including positive and negative controls. At least one Positive Control DNA (DNA 007 (2ng/μL) control, purchased separately) must be amplified with every run. At least one Negative Control consisting of 15μL of TE Buffer must also be included in every run.
2. Vortex the Master Mix and Primer Set. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.



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3. Prepare the appropriate volume of each component needed by combining the following volumes of reagents for each reaction in a microcentrifuge tube:

GlobalFiler® Master Mix: 7.5µL

GlobalFiler® Primer Set: 2.5µL

**Note:** Include at least 3 additional reactions in your calculation to provide excess volume for the loss that occurs during reagent transfer. See Appendix G (GLOBALFILER AMPLIFICATION MASTER MIX) in the Forensic Biology STR Typing Manual.

4. Mix thoroughly by vortexing.
5. Spin the tube briefly in a microcentrifuge to remove any liquid from the cap.
6. Dispense 10µL of the reaction mix into each well of the 96-well plate that will contain a negative control, positive control, or sample.
7. Add 15µL of TE Buffer into each well of the 96-well plate that will contain a negative control or sample. Add 13µL of TE Buffer to well(s) that will contain a positive control.
8. Vortex the Control DNA 007 tube (2ng/µL). Spin the tube briefly in a microcentrifuge to remove any liquid from the cap. Add 2µL of the control DNA into each well of the 96-well plate that is a positive control.
9. The final volume in each well is 25µL.
10. See BSD600 Ascent A2 protocol (preparation step 3) for next step.

#### 6.3 Specifications for GeneAmp® PCR System 9700 for GlobalFiler®

The following method for the GeneAmp® PCR System 9700 must be used for GlobalFiler® direct amplification.

**Plate type:** 96 well optical reaction plate

**Total Reaction Volume:** 25µL per well

**Ramp Speed:** Max

#### Thermal Cycler PCR Parameters for GlobalFiler®:

**Initial Incubation Step:** Hold 95 C for 1 minute

**27 cycles**

**Denature:** 94 C for 10 seconds

**Anneal/Extension:** 59 C for 90 seconds

*Note: Denature and Anneal/Extend are considered one cycle.*

**Final Extension:** 60 C for 10 minutes

**Final Step:** Hold at 4 C



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## 7.0. Protocol for Applied Biosystems 3500xL Genetic Analyzer Using Data Collection Software v.4.0.1 with GlobalFiler® for Databasing

Refer to the Forensic Biology STR Typing Manual, section *8.0 Protocol for Applied Biosystems 3500 Genetic Analyzer Using Data Collection Software v.4.0 with GlobalFiler® or Yfiler® Plus* for software and instrument set-up, preparation for and running on a 3500/3500xL Series Genetic Analyzer.

### 7.1 Special notes for 3500xL:

**Note 1:** As with the 3500 each well that will be injected to the capillary, must contain fluid; for the 3500xL this is 24 capillaries for each injection instead of 8 per injection.

**Note 2:** In the Library, the Assay (Instrument Protocol and QC Protocol), File Name Convention, and Results Group are designated under the name *GlobalFiler DB* with the following settings:

16 50 0037 1.7 PPT 3.1 01.0 1.0037

Edit Assay GlobalFiler DB

Setup an Assay

Assay Setup Help ?

\* Assay Name: GlobalFiler DB ☐ Locked Color: Dark Red

Application Type: HID ☐ Disable Filters

Protocols

Do you wish to assign multiple instrument protocols to this assay? ☒ No ☐ Yes

\* Instrument Protocol: GlobalFiler DB Edit Create New

\* QC Protocol: GlobalFiler DB Edit Create New

Close Save



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Edit Instrument Protocol GlobalFiler DB

Setup an Instrument Protocol

Instrument Protocol Setup Help ?

Application Type:  Capillary Length:  cm Polymer:

Dye Set:  ☐ Disable Name Filter

Instrument Protocol Properties

\* Run Module:  Run Modules for 24 capillary are only available in the list.

\* Protocol Name:  ☐ Locked

Description:

Oven Temperature (°C):  Run Voltage (kVolts):  PreRun Voltage (kVolts):  Injection Voltage (kVolts):

Run Time (sec.):  PreRun Time (sec.):  Injection Time (sec.):  Data Delay (sec.):

▼ Advanced Options

Following values are not recommended to be changed.

Voltage Tolerance (kVolts):  Voltage # of Steps (nk):  Voltage Step Interval (sec.):

First Read Out Time (ms):  Second Read Out Time (ms):

Normalization Target:  Normalization Factor Threshold Min:  Normalization Factor Threshold Max:

Close Save



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Edi QIC Protocol GlobalFiler DB

Setup a QC Protocol

Protocol Name: GlobalFiler DB ☐ Locked

Description: GS600LZ (50-460)

Size Standard: GS600\_LIZ\_000-460

Size Caller: SizeCaller v1.1.1

Analysis Settings: QC Settings

Analysis Range: Full Sizing Range: Partial Size Calling Method: Local Southern

Analysis Start Point: 0 Sizing Start Size: 60

Analysis Stop Point: 1000000 Sizing Stop Size: 460

Peak Amplitude Threshold: Blue 175 Green 175 Yellow 175 Red 175 Orange 175 Purple 175

Common Settings:

Use Smoothing: Light Polynomial Degree: 3

Use Baseline (Baseline Window (Pts)): 33 Slope Threshold Peak Start: 0.0

Minimum Peak Half Width: 2 Slope Threshold Peak End: 0.0

Peak Window Size: 13

Close Save

Edi QIC Protocol GlobalFiler DB

Setup a QC Protocol

Protocol Name: GlobalFiler DB ☐ Locked

Description: GS600LZ (50-460)

Size Standard: GS600\_LIZ\_000-460

Size Caller: SizeCaller v1.1.1

Analysis Settings: QC Settings

Size Quality:

| Fail If Value Is | Flagged Range | Pass If Value Is |
|------------------|---------------|------------------|
| < 0.25           | 0.25 - 0.75   | > 0.75           |

Broad Peak:

Activate Broad Peak Flag if value >: 1.5

Close Save



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Edit File Name Convention GlobalFiler DB

Setup a File Name Convention

\* Name: GlobalFiler DB ☐ Locked Color: Dark Red

Select File Name Attributes

Preview of File Name: <Sample Name>\_<Capillary Number>\_<Well Position>\_<Injection Number>\_<Date of Run>

Available Attributes

- Amplicon Name
- Analysis Protocol Name
- Assay Name
- Custom Text1
- Custom Text2
- Custom Text3
- Instrument Name

Delimiters

Select a delimiter: Underscore ( \_ )

☐ Add between attributes

Add >>

<< Remove

Move Up

Move Down

Selected Attributes

- Sample Name
- Underscore ( \_ )
- Capillary Number
- Underscore ( \_ )
- Well Position
- Underscore ( \_ )
- Injection Number
- Underscore ( \_ )
- Date of Run

Add a custom value to available attributes (optional)

Custom Text 1: Custom Text 2: Custom Text 3:

Select File Location

☒ Default File Location D:\Applied Biosystems\3500\Data

☐ Custom File Location Browse...

Close Save



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Edit Results Group GlobalFiler DB

### Setup a Results Group

Name: GlobalFiler DB ☐ Locked Color: Dark Red

Select Results Group Attributes

Preview of Results Group Name: <Plate Name>\_<Assay Name>

Available Attributes

- Injection Number
- IP Name
- Delimiters

Select a delimiter: Dash (-) ☐ Add between attributes

Selected Attributes

- Plate Name
- Underscore ( \_ )
- Assay Name

Enter a custom value as either the Prefix or Suffix (optional)

Prefix:  Suffix:

Select Reinjection Folder Option

☐ Store reinjection sample files in a separate Reinjection folder (same level as Injection folders)

☒ Store reinjection sample files with original sample files (same level)

Select Folder Option

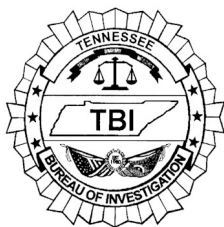
☒ Default file location D:\Applied Biosystems\3500\Data\<IR Folder>\<Plate Name>\_<Assay Name>\

☐ Custom file location  

☒ Include an Instrument Run Name folder

☒ Include a Result Group Name folder

☐ Include an Injection folder



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## **8.0. Procedure for using GeneMapper ID-X Analysis Software Version 1.6 for Analysis of AB 3500xL Data for GlobalFiler® for Databasing**

See the Forensic Biology STR Typing Manual section 9.0 *Procedure for using GeneMapper ID-X Analysis Software Version 1.6 for Analysis of AB 3500 Data for GlobalFiler® or Yfiler® Plus* for the procedure to use of the Applied Biosystems GeneMapper™ ID-X Software (GMID-X).

- 8.1 For Databasing, Once the samples have been imported, edit and fill down the following columns as follows:

**Analysis Method:** AT – Databasing

**Panel:** GlobalFiler\_Panel\_v1.1.1X

**Size Standard:** GS600\_LIZ\_(60-460)

**Sample Type:** Label Allelic Ladder, Positive and Negative Control  
appropriately

Project name should be the same as the plate #.

- 8.2 All samples, controls, and allelic ladders shall be analyzed at the analytical threshold.

1. The following thresholds apply to all dye channels. The orange (LIZ) dye channel is recommended to be analyzed at the stochastic threshold (445 RFU), but may be the analyzed at analytical threshold (175 RFU) if necessary. LIZ peaks not meeting the stochastic threshold may indicate an injection or instrument issue.

Thresholds for GlobalFiler® Databasing:

Analytical Threshold

Stochastic Threshold

175 RFU

445 RFU



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- The screenshot below provides the appropriate settings for the analytical threshold analysis method for GlobalFiler® Databasing:

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Detector' tab selected. The settings are as follows:

- Peak Detection Algorithm:** Advanced
- Ranges:**
  - Analysis:** Full Range (dropdown)
  - Sizing:** Partial Sizes (dropdown)
  - Start Pt:** 0
  - Stop Pt:** 10000
  - Start Size:** 60
  - Stop Size:** 460
- Smoothing and Baseline:**
  - Smoothing:** ☒ Light (radio buttons for None, Light, Heavy)
  - Baseline Window:** 33 pts
- Size Calling Method:**
  - ☐ 2nd Order Least Squares
  - ☐ 3rd Order Least Squares
  - ☐ Cubic Spline Interpolation
  - ☒ Local Southern Method
  - ☐ Global Southern Method
- Peak Detection:**
  - Peak Amplitude Thresholds:**
    - B:** 175 **R:** 175
    - G:** 175 **P:** 175
    - Y:** 175 **O:** 445
  - Min. Peak Half Width:** 2 pts
  - Polynomial Degree:** 3
  - Peak Window Size:** 13 pts
  - Slope Threshold:**
    - Peak Start:** 0.0
    - Peak End:** 0.0
  - Normalization:** ☐ Use Normalization, if applicable

Buttons at the bottom: Save As, Save, Cancel, Help. A 'Factory Defaults' button is also present.



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3. The Allele tab shows the marker specific stutter filter for GlobalFiler® Databasing will be set to the 20% global filter as shown below.

Analysis Method Editor

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

Bin Set: AmpFLSTR\_Bins\_v6X

☒ Use marker-specific stutter ratio and distance if available

| Marker Repeat Type:           |      | Tri  | Tetra | Penta | Hexa |
|-------------------------------|------|------|-------|-------|------|
| Global Cut-off Value          |      | 0.2  | 0.2   | 0.2   | 0.2  |
| MinusA Ratio                  |      | 0.0  | 0.0   | 0.0   | 0.0  |
| MinusA Distance               | From | 0.0  | 0.0   | 0.0   | 0.0  |
|                               | To   | 0.0  | 0.0   | 0.0   | 0.0  |
| Global Minus Stutter Ratio    |      | 0.0  | 0.0   | 0.0   | 0.0  |
| Global Minus Stutter Distance | From | 2.25 | 3.25  | 4.25  | 5.25 |
|                               | To   | 3.75 | 4.75  | 5.75  | 6.75 |
| Global Plus Stutter Ratio     |      | 0.0  | 0.0   | 0.0   | 0.0  |
| Global Plus Stutter Distance  | From | 0.0  | 0.0   | 0.0   | 0.0  |
|                               | To   | 0.0  | 0.0   | 0.0   | 0.0  |

Amelogenin Cutoff 0.0

Range Filter... Factory Defaults

Save As Save Cancel Help



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4. The following screenshot provides the recommended Peak Quality settings for GlobalFiler® Databasing:

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Quality' tab selected. The dialog contains several sections for configuring peak quality parameters:

- Min/Max Peak Height (LPH/MPH):**
  - Homozygous min peak height: 445.0
  - Heterozygous min peak height: 445.0
  - Max Peak Height (MPH): 25000.0
- Peak Height Ratio (PHR):**
  - Min peak height ratio: 0.6
- Broad Peak (BD):**
  - Max peak width (basepairs): 1.5
- Allele Number (AN):**
  - Max expected alleles:
    - For autosomal markers & AMEL: 2
    - For Y markers: 1
- Allelic Ladder Spike:**
  - Spike Detection: Enable (dropdown)
  - Cut-off value: 0.2
- Sample Spike Detection:**
  - Spike Detection: Enable (dropdown)

At the bottom right of the dialog is a 'Factory Defaults' button. At the bottom of the dialog are four buttons: 'Save As', 'Save' (highlighted in blue), 'Cancel', and 'Help'.



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5. The following screenshot provides the recommended SQ and GQ Settings for GlobalFiler® Databasing:

Analysis Method Editor

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

Quality weights are between 0 and 1.

Sample and Control GQ Weighting

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

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

SQ Weighting

Broad Peak (BD) 0.5

Allelic Ladder GQ Weighting

Spike (SSPK/SPK) 1 Off-scale (OS) 1

SQ & GQ Ranges

|                   | Pass Range:      | Low Quality Range: |
|-------------------|------------------|--------------------|
| Sizing Quality:   | From 0.75 to 1.0 | From 0.0 to 0.25   |
| Genotype Quality: | From 0.75 to 1.0 | From 0.0 to 0.25   |

Reset Defaults

Save As Save Cancel Help



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#### **8.3 Printing Samples**

1. For GlobalFiler® Databasing samples, artifacts and micro-variants should be documented within GMID-X to ensure that the CODIS export file is correct. If a change is made to a sample after the packet stop date, the change should be made in GMID-X and documented in the packet.
2. The Label Edit Viewer may also be printed and included in the packet at the analyst's discretion.
  - a. Save the project.
  - b. Select samples with edits.
  - c. From the Project window, select view > Label Edit Viewer > Export
  - d. Save the Export as a .csv file.
  - e. Open the .csv file in Excel and print as a pdf.
3. Electropherograms shall be analyzed and printed at the analytical threshold.
4. All electropherograms will be printed with the allele calls. The electropherograms must also include peak heights except for the allelic ladders. Peak sizes may also be included at the analyst's discretion. It is recommended to print at least one allelic ladder with the base pair sizes if an allele designation has been manually calculated for an off-ladder peak.

#### **8.4 Exporting CODIS data for import by SDIS**

Profiles shall be technically reviewed by a qualified analyst prior to exporting the data.

1. In the Project window, change the Table Settings to *CODIS Export*.
2. Assign *Specimen Category*.
  - a. Failed samples, positive controls, negative controls, and ladders leave category as *No Export*.
3. Save the project.
4. File > Export Table for CODIS.
5. Export as:
  - a. File name: Plate# CODIS Import File
  - b. CMF 3.3 (.xml)
  - c. Source: TN0792500
  - d. Destination: TNTBI0000



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6. Select *Export*.
7. Save file to CODIS Import Files folder on the L drive.
8. Complete the grant tracking log file with the plate#, db#s, date export file added to folder

#### **8.5 Exporting GMID-X Projects**

1. This should be done after the review of the data is complete.
2. In the Project window, select the *GeneMapper ID-X Manager* icon.
3. Under the *Projects* tab, select the project, then select *Export...*
4. Save the *.ser* file to the Databasing GMID-X Projects archive on the L drive and backed up on the analyst's external hard drive.

#### **8.6 Importing GMID-X Projects**

1. In the Project Window, select the *GeneMapper ID-X Manager* icon.
2. Under the *Projects* tab, select *Import...*
3. Select the *.ser* file and ensure *Skip* is selected (same analysis settings already exist in the software).
4. Select *Import*.
5. File > Open (or Open icon) > select project > OK.



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## **9.0. Assessment of GlobalFiler® Databasing Data**

See the Forensic Biology STR Typing Manual section *10.0 Assessment and Reporting of GlobalFiler Data* for the assessment of data and identification of alleles and artifacts.

**Note:** If more than one positive or negative control is run on the plate, at least one positive and one negative control shall type correctly for the run data to be interpreted. All controls run shall be printed and included in the packet, controls that do not pass shall be marked Data Not Used with the analyst's initials, date, and reason for rejection of the data. If no positive or negative control run with the plate passes, then all samples amplified at the same time shall be re-punched. If re-punching is not possible for some samples, re-collection of the sample should be requested.

For Databasing, the 3500xL instrument is with the J6-OSR spectral, resulting in an extended dynamic range of 3,000 to 25,000 RFU and off-scale peaks recovered up to 65,000 RFU.

The data collected from an ABI 3500xL will be analyzed with GeneMapper ID-X v1.6.

### **9.1 Database Sample:**

A database sample determined to have contamination must be re-punched or processed with the Forensic Biology workflow, if sample size permits. If the contamination is still present, then a recollection request must be sent. In instances where sample size does not permit re-testing of potentially contaminated samples, then a recollection request must be sent. The DNA Technical Leader must be notified and this will be documented and retained in the database file.

Profiles not meeting quality control standards due to failed controls and/or contamination may be deemed uninterpretable. Electropherograms for uninterpretable profiles should be clearly labeled as not being used for interpretation. If possible, these samples should be re-processed to correct any quality control issues.

### **9.2 GlobalFiler® Databasing thresholds for Direct Amplification:**

The analytical threshold (AT) is set at 175 RFU for all dye channels on a 3500xL.

The stochastic threshold (ST) is set at 445 RFU for all dye channels on a 3500xL.

For database samples, data between the analytical and stochastic thresholds may be used. If a single allele is present between the analytical and stochastic thresholds, the sample should be re-injected or re-processed, see Appendix B for the databasing workflow.



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## **Appendix A**

### **Databasing Critical Reagents**

These reagents must be quality control checked prior to use.

TE Buffer

GlobalFiler® Amplification Kit:

GlobalFiler® Master Mix

GlobalFiler® Primer Set

GlobalFiler® Allelic Ladder

DNA Control 007 (2ng/μL)

For samples and testing following Forensic Biology procedures, for a list of the associated critical reagents see Appendix B in the Forensic Biology STR Typing Manual.



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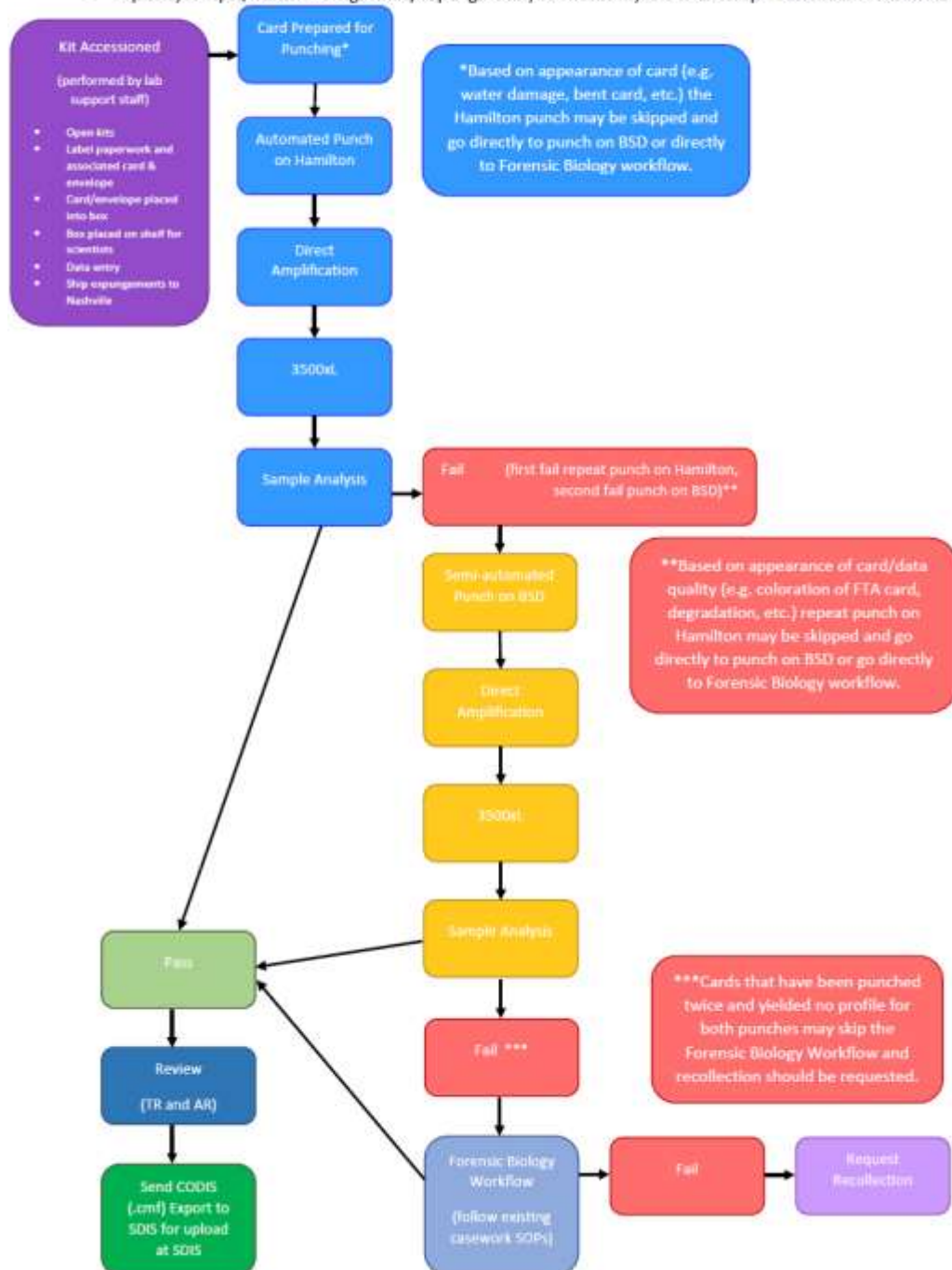
### CODIS Databasing Manual

## Appendix B

### Databasing Workflow

#### Workflow:

- Accessioning through Review in Jackson Lab.
  - o Card prepared for punching through Review performed by a qualified analyst.
- Upload, sample/match management, expungement, verifications, and final sample retention in Nashville I.





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## Appendix C

### Packet Assembly

Packets will typically be named by plate #. The plate # will typically consist of the start date of the run and analyst initials (e.g. 06082024\_KDM). If an analyst has multiple runs started on the same day, a unique identifier shall be added to the name (e.g. \_1 and \_2 or \_BSD). If a supplemental or amended packet needs to be created, the original plate # should be used along with an indicator such as \_S for supplemental and \_A for amended.

#### Worksheet Order:

1. Stamp Page – Databasing
2. QC Page – Databasing
3. Punch and Direct Amp Plate – Databasing
4. CE Plate – Databasing
5. Electropherograms

**Note:** Database samples processed following the Forensic Biology workflow should utilize the relevant worksheets and worksheet order described in the *Forensic Biology and CODIS QA Manual*, Appendix A. Database samples processed as verifications will follow the Jackson lab databasing workflow but will also include the completion of the necessary paperwork and reviews according to the *CODIS Unit Manual*, section 12.3 *Verification of an Offender/Arrestee Hit*.

#### Commonly Used Abbreviations in Packets and GMID-X:

(additional abbreviations can be found in the *Forensic Biology Manuals* referenced in Appendix E)

|     |                                                      |
|-----|------------------------------------------------------|
| ART | ARTIFACT                                             |
| CN  | CONFIRMED NULL ALLELE(S)                             |
| CT  | CONFIRMED TRI-ALLELE(S)/TRISOMY                      |
| DNU | DATA NOT USED                                        |
| EA  | ELEVATED ARTIFACT(S)/ADDITIONAL PEAK(S) OR ALLELE(S) |
| MP  | MIXTURE PROFILE                                      |
| MV  | MICRO-VARIANT                                        |
| NE  | NO EXPORT                                            |
| NP  | NO PROFILE                                           |



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|     |                                                 |
|-----|-------------------------------------------------|
| NSD | NO SIZING DATA                                  |
| NTP | NOT PUNCHED                                     |
| PM  | PEAK MORPHOLOGY                                 |
| PP  | PARTIAL PROFILE                                 |
| RI  | RE-INJECTION                                    |
| RIA | RE-INJECTION DUE TO POSSIBLE ARTIFACT           |
| RIL | RE-INJECTION DUE TO LOW RFUs                    |
| RIS | RE-INJECTION DUE TO SIZE QUALITY/NO SIZING DATA |
| RP  | RE-PUNCH                                        |
| TRI | POSSIBLE TRI-ALLELE(S)/TRISOMY                  |

**Note:** No pages should be deleted from a file after the packet is given a stop date.  
Pages may be added as required for each packet.



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## **Appendix D**

### **Packet Review**

#### **Technical Review:**

- Shall be conducted by an analyst or technical reviewer qualified in the method, technology, typing test kit, platform, and interpretation software being reviewed having completed a competency test prior to participating in the technical review of DNA data. Each technical reviewer will participate in an external proficiency testing program on the same technology, platform, and typing amplification test kit used to generate the DNA data being reviewed.
- The technical review shall include:
  - A review of all notes and worksheets.
  - A review of the electronic project and printed electropherograms, including allele edits and specimen category designations, that support the results and conclusions for conformance with proper procedures and policy. This review includes verifying calculations and data transfers.
  - A review of all DNA types to verify that they are supported by the raw or analyzed data.
  - A review of all controls, internal lane standards, and allelic ladders to verify that the expected results were obtained.
  - The completion of the technical review is documented on the first page of the packet with the reviewer's initials and date of review completion.

#### **Administrative Review:**

- Shall be conducted by an individual who is not the author of the report and is a currently or previously qualified analyst.
- The administrative review shall include:
  - A review of the packet for clerical errors including spelling and grammatical accuracy and that information specified in Appendix C is present and accurate.
  - A review of all records to ensure that the records are uniquely identified with the plate number(s), analyst's initials (or electronic equivalent), and page numbers.
  - The completion of the administrative review is documented on the first page of the packet with the reviewer's initials and date of review completion.

#### **Packet Review:**

- Packets and records of observations are subject to subpoena or discovery and must be of a permanent nature. Corrections are to be made by a single line strike-through with



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the initials of the person making the change. Contemporaneous changes, alterations, and additional notations (including interlineations) made in packets must include the initials of the person making the additions. If changes, additions, or corrections are made to the packet after the stop date, the change, addition, or correction must include the date and the initials of the scientist or person responsible.

- If a review worksheet, summary of comments, or other administrative documentation is completed for a packet, this should be included before the stamp page, at the end of the packet, or attached to the packet. Administrative documents do not require page numbers.



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## **Appendix E**

### **REFERENCES**

#### **PUNCHING SYSTEMS**

Hamilton Bonaduz AG Hamilton EasyPunch Operator's Manual

Hamilton Bonaduz AG Hamilton STARlet Operator's Manual

Hamilton Bonaduz AG Hamilton VENUS Software Programmer's Manual

BSD Robotics BSD600 Ascent A2 User Manual

#### **AMPLIFICATION**

Gene Amp PCR System 9700 User's Manual

GlobalFiler and GlobalFiler IQC PCR Amplification Kits User Guide

TECHNICAL NOTE: Direct Amplification of Reference Samples Using the GlobalFiler™ PCR Amplification Kit

#### **STR ANALYSIS**

Applied Biosystems 3500 / 3500xL Genetic Analyzer User Guide

Genemapper ID-X Software version 1.0 Getting Started Guide

Genemapper ID-X Software version 1.6 User Bulletin

#### **TBI POLICY**

CODIS Unit Manual

Forensic Biology Policy and Procedures Manual

Forensic Biology and CODIS QA Manual

Forensic Biology STR Typing Manual

For each manual listed above, the current revision in use is available in the document control system (Ensur and/or PowerDMS).