28.1 PURPOSE

Processing Convicted Offender/single source samples (SSS), comprehensively from setting up worksheets and plates/tubes, and amplifying samples. Additionally, the interpretation of and import into CODIS of database samples.

28.2 RESPONSIBILITY

Forensic Science Examiners 1, 2, and 3 and Laboratory Assistants 2 in the DNA Unit.

28.3 Database plate sample list preparation (of consecutive database samples)

28.3.1 Use #1-1 database cards for all samples, except QC samples which can be either #1-1 or #1-2.

28.3.2 Determine the range of samples to be tested (all must be consecutive, except QC samples). Two samples must be QC samples. Take the QC sample cards with you to the LIMS computer.

28.3.3 In LIMS, select the "Crystal Reports" icon.

28.3.4 Choose "DNA Database Plate Sample List", then Print, and Screen.

28.3.5 With "Starting Sample" highlighted, enter the first sample number in the format specified. Next highlight "Ending Sample" and enter the last sample number in the same way. Select OK.

28.3.6 Select the Export Report icon in the upper left. For Format, choose tab-separated text; for Destination, choose Disk File. Select OK. Name the file and save.

28.3.7 Open the file just saved using Notepad. To do this:

28.3.7.1 Select the file. At the first screen, choose to select the program to use from a list. Do not use the web service.

28.3.7.2 At the second screen, uncheck the box next to "always use the selected program to open this kind of file."

28.3.7.3 Select Notepad from the list and OK.

28.3.8 At two distant positions from each other in the list, add a line for each QC sample. At each position type the QC sample number, hit tab, and scan the barcode.

28.3.9 Save the file on a removable disk or shared drive.

28.3.10 On a computer with U-Drive access, open the file using Microsoft Excel. Copy all the data in the two columns.

28.3.11 Open the Direct Amplification Worksheet (QR-29). In the LIMS tab, paste into cell A2 all the data copied in 28.3.10. Continue to 28.6.

28.4 Database plate sample list preparation (of non-consecutive database samples)

28.4.1 Collect all the database cards to be tested (#1-1 and/or #1-2). Swabs may be run on the database plates, but must be manually entered onto DNA QR-29 (Fusion 6C Direct Amp Worksheet- DB).

28.4.2 Bring the cards to a computer with a barcode scanner. Open Microsoft Excel. Type the number of the first database sample in cell A01, in DB-XX-XXXXXX format. In cell B1, scan the barcode on this card.

28.4.3 Continue to enter the database numbers into column A and the corresponding barcodes into column B, for all samples including QC samples, in the order of testing. Note: If the plate includes Database Hit Confirmations, no QC samples are required.

28.4.4 Save the file on a removable disk or shared drive.

28.4.5 On a computer with U-drive access, open the file using Microsoft Excel. Copy all data in the two columns.

28.4.6 Open the Direct Amplification Worksheet (QR-29). In the LIMS tab, paste into cell A2 all the data copied in 28.4.5. Continue to 28.6.

28.5 Plate workbook setup

28.5.1 Note: Detailed instructions on plate workbook setup (full and partial plates for database and casework samples) can be found in the Database and Known casework sample worksheets: QR-29.

28.5.2 Naming of Database Plates:

28.5.2.1 Every plate will have a letter and numerical designation. The letter will be indicative of the type of lab work completed on the plate, and the numerical designation will be indicative of when the testing took place, whether it is a date or a consecutive counting.

28.5.2.2 Letter Designation:

i. **E**: First Amplification
ii. **E2**: 2nd Amplification
iii. **A**: Swab Amplification
iv. **R**: Reinjection
v. **AR**: Plate containing both reamplifications and reinjections
vi. **HC**: CODIS hit confirmations
vii. **HC-E2**: Plate containing both CODIS hit confirmations and 2nd extractions
viii. **PT**: for Proficiency Tests

28.5.2.3 Numerical Designation:

i. **For E plates only**: the plates will be numbered in order based on the year and sequence the samples were received at the laboratory. For example, the 50th plate of samples that came into the laboratory in 2010 would be named E-2010-50.
ii. **For all other plates**: the numerical designation will be a 6 digit number corresponding to the date that lab work was **started** on those samples. For example, for an A plate, if the quant was done on September 1st, 2011 the amplification on September 2nd, 2011, and the injection on September 5th, 2011, the plate name will be A-090111.

28.5.2.4 This unique identifier must be written on the top right of every paper associated with the lab work completed for this plate, and will be considered a means of batching a group of database samples with one specified name.

28.5.2.5 If a situation presents itself that is not listed, please follow the logic behind the protocols in keeping the naming consistent.

28.6 Punching a plate using the BSD Duet

NOTE: Detailed instructions can be found in Appendix I of this SOP and QR-29.

28.6.1 Turn on the BSD Duet.

28.6.2 Design the program to accommodate all the samples and controls.

28.6.3 Use the barcode reader when applicable.

28.6.4 Punch all the samples and controls, ensuring samples were placed in the correct wells.

28.6.5 Turn off the BSD Duet when done.

28.6.6 When necessary, clean and perform maintenance on the BSD Duet as detailed in Appendix II of this SOP.

28.7 Manual Punching of FTA Cards

28.7.1 FTA cards may be punched manually; this is generally only appropriate if the number of cards to be tested is small.

28.7.2 Ensure that the DB or ID number on the laboratory barcode or on the foil envelope matches the sample number to be tested.

28.7.3 FTA cards are punched with a **3mm** punch into tubes for extraction using SOP-19 (with Identifiler Plus amplification) or SOP-20 (with Identifiler Plus or Fusion 6C amplification). Alternatively, FTA cards are punched with a **1.2mm** punch (either by hand or on the punchbot) into tubes or 96 well plates for Fusion 6C Direct Amplification, see (SOP-26).

28.7.4 After punching each sample or control, clean the tip of the puncher by punching a disc out of clean filter paper or a clean FTA card.

28.8 Amplification of SSS Using Fusion 6C Direct Amplification

NOTE: See SOP 26: Fusion 6C Direct Amplification and Detection

28.10 Analysis of database samples

28.10.1 After injecting a set of samples on a 3130 (see SOP 26), create a folder in "U:\Database no suspect unit\convicted offender data\being analyzed" with your plate name. Copy the Data Set run folders from the 3130 computer to the folder just created.

28.10.2 The analyst shall analyze the data using peak height thresholds of 50 RFU. Routine click-offs do not need to be noted on the analysis worksheets. The positive control(s) DNA profile(s) are checked using QR-45 (A, D, E, F) QR-49A, E (POS and EP1 Concordance Checkers) or QR-37 (GeneMarker Concordance Check). Allele changes and sizes of peaks out of ladder ranges should be noted in the comment section of the comment chart (QR-35 Fusion 6C Injection Worksheet (14h)), as should any low peak height ratios and possible tri-alleles. Change out of bin (OB) alleles to their true allele designations as appropriate, making sure the allele is accepted at CODIS. Any alleles that are not accepted at CODIS should be changed to a <# or # >. The # indicates the minimum/maximum allele acceptable at CODIS for that locus (i.e. FGA 31.2 is changed to >30). The Known PHR Filter Macro is used to assist analysts in identifying any results warranting further review or documentation.

28.10.3 For incomplete or unacceptable profiles, determine if the sample needs reamplified, the swab reamplified or reinjected. If applicable, check the appropriate box on the comment chart. If a partial profile is obtained, print out this profile (for comparison purposes to the full profile (when obtained)). Disable these incomplete or unacceptable profiles from the project. Fill out the "Database Quant and Reinject" spreadsheet located on the U-drive. Include the sample name, well number, reason for re-do, and all appropriate controls to be redone with the sample.

28.10.4 For database known processing, the RB and the Neg must be amplified and injected to reflect the largest amplification volume and the longest injection times for the samples associated with them.

28.10.5 If called peaks arise in the RB or the Neg on database plates, interpret with caution, and see DNA Technical Leader for approval/documentation of the control. If the CODIS administrator does not approve the control it will be necessary to re-amplify affected samples.

28.10.6 If after re-injection, and/or re-amplification, a complete DNA profile has not been generated, the applicator sponge/swab should be used for Amplification. This envelope containing the sponges has a temporary seal (as per DNA SOP-11) and will be disposed after a complete DNA profile has been generated. If the sponge fails to generate a full DNA profile, a copy of the identifying information for the offender sample will be made

and delivered to the CODIS Administrator. The CODIS Administrator will notify the submitting agency that a new sample needs to be collected from the offender.

28.10.7 For acceptable profiles, on the comment chart (QR-35 (14h)), check the box for good profile (if applicable). Save and export the project to the folder created in step 28.10.1. The SGF file and the plate name should be the same.

28.10.8 The Technical Reviewer shall import the GeneMarker file (.SGF) into GeneMarker. The Technical Reviewer should review the data, but not make any changes. Comments or questions should be noted-and given to the primary examiner and maintained on the appropriate QR worksheet. There is no need to save or export this file.

28.10.9 The 1st analyst shall review and/or make changes that the 2nd analyst suggested. If changes were made, save and export the file again, overwriting the original SGF file.

28.10.10 The Technical Reviewer shall technically review all paperwork associated with analyzed samples. These checks will be noted on QR-35.

28.11 Importing database samples into CODIS

28.11.1 Move file folder created in step 28.10.1 to U:\Database-nosuspect unit\Convicted offender data\completed folder.

28.11.2 Insert a removable disk into the computer.

28.11.3 Open GeneMarker and the project to be imported into CODIS. Select Application and Export CODIS

28.11.4 Change Source ORI and Destination ORI to CTCSP3500

28.11.5 Select PowerPlex Fusion 6C as PCR kit

28.11.6 Fill out Submit User ID: Using your CODIS User ID

28.11.7 Select CMF 3.2 (.xml) file

28.11.8 The default setting is to have the check box selected for export. Verify that controls, ladders, and samples not to be exported are not selected. Deselect any specimens as necessary.

28.11.9 The default setting for Specimen Category is Convicted Offender. Verify the Specimen Category is set to Convicted Offender for each sample to be exported.

28.11.10 Click OK

28.11.11 Save and export the file to a removable disk.

28.11.12 On a CODIS computer, insert the removable disk, then open the Analyst Workbench.

28.11.13 On the lower left-hand bar, select Specimen Manager.

28.11.14 On the tool bar, select Specimen Manger and Import. Alternatively, select the Import icon.

28.11.15 Browse for the file created in step 28.11.11

28.11.16 Select Import Type as Data Import and assign the user to you, then select OK.

28.11.17 On the confirmation message that input file was successfully sent to the message center, select OK.

28.11.18 On the lower left-hand bar, select Message Center. Above that bar, Import STR Files should be in bold. This indicates your file successfully was sent to the message center.

28.11.19 Select Import STR Files. You should see the file that you just imported in the larger window. Double-click on that file.

28.11.20 When the file has finished importing, Import Reports on the left side should be in bold.

28.11.21 Select Import Reports and choose the file that was just created. An SDIS Import Reconciliation Report should appear on the screen. Review of this report will ensure the samples have been imported and assigned the correct CODIS specimen category.

28.11.22 Verify that the correct QC samples were imported and concordant with previous entries. Verify that the number of new database samples being imported is correct. Verify that all

newly imported samples are assigned the correct specimen category. Document the verification on DNA QR-35.

28.11.23 Print out the first page of the import reconciliation report and attach it to DNA QR-35.

28.11.24 Any problems listed on the SDIS Import Reconciliation Report must be rectified.

28.11.25 File away with appropriate database paperwork.

28.11.26 Update the In-House Database Post-Processing spreadsheet for each sample that a good profile was obtained. Fill in the CT Plate Name and Date Sample Uploaded to CODIS columns.

28.12 Searching for duplicate specimens

28.12.1 On a CODIS computer, open Analyst Workbench and click on AutoSearcher on the lower left-hand bar.

28.12.2 Under Identity Search, click on Duplicate Offenders. Click on the Perform Search icon. The Duplicate Offender tab should open. If any duplicates were found, it will say so in the message text.

28.12.3 Click on Match Manager on the lower left-hand bar. The new matches that were just found in AutoSearcher should be listed in red at the top of the default view. Highlight the matches, click the Print icon, and Match Inventory Report. Make sure that the Target Specimen ID and the Candidate Lab ID columns both print. If you need to, you can adjust the column width in the default view.

28.12.4 Find the offender information cards for both the target specimen and candidate specimen, and assure the sample is in fact a duplicate through comparison of the identifying information. If the sample is a duplicate, proceed to 28.13. If the sample is not a duplicate, identify the issue(s) that has caused the sample to be matched as duplicates. Notify the CODIS Administrator for appropriate match disposition and specimen category assignment. If ambiguities continue to exist the CODIS Administrator will forward the match/information to the DNA Data Bank Oversight Panel for resolution.

28.13 Disposition of duplicate samples in CODIS

28.13.1 On a CODIS computer, open Analyst Workbench and click on Match Manager on the lower left-hand bar.

28.13.2 Find the sample(s) that you have determined to be duplicate sample(s). Highlight one or all the samples. Right click, scroll down to Set Disposition then Set Own State's Specimen Disposition as Offender Duplicate.

28.13.3 Click on Specimen Manger on the lower left-hand bar. Find the samples that were just dispositioned in Match Manger. Highlight one or all samples. Right click, scroll down to Set Specimen Properties then Specimen Category. From the drop-down menu, choose CO Duplicate and click OK.

28.13.4 Close out of the Analyst Workbench. There is no need to save anything.

APPENDIX I: Punching samples on the BSD Duet:

Turning on the BSD Duet

1. In any order, start the computer and log on, turn on the BSD Duet using the switch on the right side, and turn on the air pump by plugging it in.

2. Double click the BSD 600 Menu icon to open BSD Duet software. Log on.

3. Click on Configure System and the Files tab. Select Input File Name by browsing to the file that was previously saved to a removable disk or shared drive. Set Sample Number Mode field to File Input.

4. Go to the Barcode tab. Check the box next to Samples (unless you are not using barcodes); the rest of the boxes should be unchecked. Click Save and Exit. (If you are punching a full database plate and working from the existing full plate test, proceed to the section: Punching a Full Plate. If you are punching a partial database plate or a known SSS plate and need to create a test, proceed to the section: Creating a Test for a Partial Plate.

Creating a New Test

1. Before beginning, it is helpful to make a plate map on a worksheet if you have not yet done so.

2. Click the Edit Test Sequences box in the BSD Main Menu to open the Test Editor program.

3. Choose Create a new test.

4. Choose Microtitre.try as the type of tray.

5. From the Test pull down menu, select Test Configuration. Click the Automatic filling tab and change the Fill Direction to vertical. Click the Spot per cell tab and choose the number (up to 6) and size (1.2mm) of spots to be placed in cells. Click OK.

6. Choose a cell type for each cell of the tray. Double-click on a single cell to do this, or make a box around a group of cells and right-click to change them all to the same type.
   a. Samples (including QCs) = Sample
   b. RB and EP1 = Control
   c. Ladder, Neg, and Pos = Liquid Control, or Unused Cell
   d. Blank = Unused Cell

7. Control Usage for Single Source Samples:
   a. For Database known samples, 1 RB, 1 Neg, and 1 Pos will run on a plate. 3 EP1s will run on a full plate, and at least 1 EP1 will run on a partial plate.
   b. For casework known samples, 1RB, 1 Neg, 1 Pos and at least 1 EP1 will run on a plate.

8. Change the Filling Sequence Numbers to differentiate between Controls (i.e. RB vs. EP1) or Liquid Controls. (By default, automatic filling is enabled for Samples only. Automatic filling can be disabled by un-checking the box in the Automatic Filling tab of the Test Configuration box.)

9. Save your test (in BSD600 folder on local disk C) by clicking the icon or using the pull-down File menu. Give your test a name when asked. There are no naming restrictions. Delete this test after punching, unless you plan to re-use it often.

10. Close the Test Editor program using the Exit pull-down or clicking the "x" box in the upper right corner. Proceed to section: Punching a Plate.

Creating a Test for a Partial Plate

1. Open the Edit Test Sequences program. Open test DB Plate. Mark unused sample and control wells as unused by double-clicking single cells or selecting a range and right-clicking. Go to the File menu, select Save As, rename the test, and save. Delete this test after punching, unless you plan to re-use it often.

Punching a Plate

1. Open the Distribute Spots program and follow the prompts on the program. For a full plate, choose "DB plate" as the test to punch. For a partial plate, choose the program created in Creating a Partial Plate, step 1 (see above). Make sure only one test is checked off. Check the boxes for Samples, Controls, and Cleaning. Remember when loading Slip-Prep plate to remove the white collar from the plate. Make sure well A01 is in the upper-left corner.

2. Before punching, intentionally scan an incorrect barcode to ensure reader is functioning properly. Document this on QR-29. Punch the plate, scanning LIMS barcodes for each sample before punching. If a barcode is incorrect, the program will pause and notify you.

3. To punch a card, slide the FTA card under the metal clamps and align the red laser dot with the spot to punch. The area punched should be stained, but isn't always. Press the pad to punch or use the automatic trigger. Between samples, use a blank card to do a cleaning punch.

4. Note: The sensor only detects the spot in the chute, not whether it actually made it into the well. For this reason click Inspect Trays and check wells often. It is easier to repunch samples before the test is finished than at the end. If spots are not falling into the center of the well or there is too much static, correct the problem by increasing the air flow in the air pump and/or add more water to the bottle attached to the Duet and air pump.

5. After punching all samples and the input file is complete, click Continue Punching to punch controls. If multiple punches of the same control are being made, multiple laser spots will appear. The Duet will punch and distribute all spots from this card at once. If you want to punch spots individually, click Shrink Pattern.

6. When controls are punched, check to see that all spots are present, and click All Spots Present and End Run.

Appendix II: Maintaining the BSD Duet

Cleaning the BSD Duet

1. The chute should be cleaned at least once every 400 punches, or 2 full plates. The cleaning should be logged on QR-12. Be careful not to inhale dust from the Duet when cleaning.

2. Unplug the air pump and turn the Duet off.

3. Open the cover and lift the card platforms. Use the black handle to rotate the punching apparatus 90 degrees to the left.

4. Unscrew the inner chute (top part of the punching apparatus) and remove. Leave the outer chute attached by the wire.

5. Clean only the inner chute using 100% ethanol and the Duet cleaning tool. Remove debris from both chutes using a can of compressed air. Reattach the inner chute to the outer chute and to the BSD Duet.

6. Move the punching apparatus back into the upright position. Direct a strong flow of compressed air under the punch guide to remove debris collected on the clamps and card holding area.

7. Check for any stray spots inside the BSD Duet and remove. If necessary, clean any dusty areas.

8. Return the card platforms and cover to their original positions. The BSD Duet and air pump can be left off or on when not in use.

BSD Duet Punchbot Performance Checking

1. Design a program (refer to section: Creating a New Test) which will use the barcode reader, punch the following sizes: 1.2mm and 3.0mm punches, and punch the following types of punches: samples, controls, standards, and cleaning.

2. Run the program.

3. Confirm the following: The barcode reader functions properly, the 1.2mm and 3.0mm punchers function properly, and that all the punches were placed in the proper locations.

4. Fill out QR-265 BSD Duet Performance Check.