18.1 PURPOSE

Guidance for processing single source samples (SSS) with Promega DNA IQ extraction for amplification with Promega Fusion 6C

18.2 RESPONSIBILITY

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

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

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

18.3.2 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.

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

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

18.3.5 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.

18.3.6 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.

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

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

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

18.3.7.3 Select Notepad from the list and OK.

18.3.8 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.

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

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

18.3.11 Open the DNA IQ Database Plate Setup Workbook (QR-14g, 14h). In the LIMS tab, paste into cell A2 all the data copied in 18.3.10. Continue to 18.6.

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

18.4.1 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 QR-14g. Swabs must be added to the deep well plate after all FTA punching has been completed.

18.4.2 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.

18.4.3 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.

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

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

18.4.6 Open the DNA IQ Database Plate Setup Workbook (QR-14g, 14h). In the LIMS tab, paste into cell A2 all the data copied in 18.4.5. Continue to 18.6.

18.5 Known casework plate sample list preparation

18.5.1 Buccal FTA samples, blood FTA samples, and buccal swab samples may all be extracted on a single DNA IQ plate. Swabs must be manually entered onto the

extraction worksheet (QR-14f). Swabs must be added to the deep well plate after all FTA punching has been completed.

18.5.2 DNA IQ may not be used to extract DNA from known liquid blood or known bloodstains on any substrate (containing blood) other than FTA paper without the approval of the Technical Leader.

18.5.3 If only buccal swab (not FTA) samples, and/or manually punched FTA samples are being extracted, proceed to step 18.6. The remainder of this procedure, starting at step 18.5.4, refers to FTA blood and buccal samples being punched using the BSD Duet.

18.5.4 In LIMS, transfer all the samples to be tested to "DNA Knowns – To Be Tested". A maximum of 84 samples can be tested.

18.5.5 Select the Crystal Reports icon.

18.5.6 Select the DNA Casework Known Plate Sample List, then Print, and Screen.

18.5.7 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 on a removable disk or shared drive.

18.5.8 Open the DNA IQ Forensic Known Plate Setup Workbook (QR-14f). In the LIMS tab, click "Import Sample List" to import the previously saved crystal report in step 18.5.7. When there is a need to develop profiles for the staff index, type in the name in the "Case #" column following imported sample cases and assign a number in the barcode column (ex. 1, 2, 3…). These numbers will be set up as manually entered barcodes when punching these samples using the BSD Duet.

18.5.9 After the macro is run, manually type in any swab samples, and/or manually punched FTA samples not being punched robotically to the "Extract and Amp" tab of QR-14f.

18.5.10 If any casework known buccal swabs (not FTA cards) or manually punched FTA cards are to be extracted using DNA IQ, fill their names into appropriate wells. A separate RB must be added to the plate for the buccal swab samples. A suggested name for this is "RBSwab" because after extraction its volume (along with that of

the genomic extracts from the swab samples) will be raised from 40µl to 200µl using dH_2O.

18.5.11 In LIMS, transfer all samples from "DNA Knowns To Be Tested" into your name.

18.6 Plate workbook setup

18.6.1 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-14g, 14h and QR-14f.

18.6.2 Naming of Database Plates:

18.6.2.1 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.

18.6.2.2 Letter Designation:

i. **E**: First Extraction
ii. **E2**: 2nd extraction
iii. **A**: Quant and Reamplification
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

18.6.2.3 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.

18.6.2.4 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.

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

18.7 Punching a plate using the BSD Duet

NOTE: Detailed instructions can be found in Appendix I of this SOP and QR-14g, 14h and QR-14f.

18.7.1 Turn on the BSD Duet.

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

18.7.3 Use the barcode reader when applicable.

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

18.7.5 Turn off the BSD Duet when done.

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

18.8 Manual Punching of FTA Cards (for DNA IQ or EZ1 Advanced XL extraction)

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

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

18.8.3 For each sample, place the FTA card face-up on the punching mat. Using a 3.0mm puncher, remove one disc from the stained area and place in a well of a Slic-Prep plate for DNA IQ extraction (see SOP-18 Appendix III for details). Alternatively, for EZ1 extraction, using a 3.0mm puncher, remove four discs from the stained area and place in an EZ1 sample tube (see SOP-19 and SOP-20 for details).

18.8.4 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.

18.9 Extracting SSS on the Biomek 3000

NOTE: Guanidine salts can form highly reactive compounds when combined with bleach. Do not use bleach to clean up reagent spills. If reagents spill, completely soak up liquid with paper towel, and then clean area with water, followed by bleach. Please also refer to the DNA IQ Users' manual.

18.9.1 Add 200µl of Lysis Buffer and 2µl 1M DTT to all sample wells, reagent blank wells, and laboratory extraction positive control wells. Wells that, in the future, will host ladders, the amplification negative and positive controls, do not need the addition of Lysis Buffer and DTT. As an option, create required amount of Lysis Buffer/DTT master mix for future addition to the troughs on the Biomek 3000.

18.9.2 Seal the plate and incubate at 70ºC for 1-2 hours.

18.9.3 After 1-2 hours of incubation, remove the plate from the incubator.

18.9.4 Slide the plastic collar into position between the top basket and the bottom plate.

18.9.5 Centrifuge the plate at 1500rpm for 5 minutes.

18.9.6 Remove the collar and top basket and discard.

18.9.7 Place the bottom plate on the Biomek 3000 deck in its corresponding position.

18.9.8 For detailed instructions on extracting SSS see AppendixIII. (Biomek 3000). In general, the process proceeds as follows:

18.9.9 Turn on the computer, the robot, and attachments.

18.9.10 Run the required start up tests.

18.9.11 Choose the appropriate DNA IQ program.

18.9.12 Setup the deck.

18.9.13 Run the program.

18.9.14 Upon completion of the program, cover the plate containing genomic DNA. Store the plate @-20°C. Clean the deck and pipette head with dH_2O.

18.10 Manual Amplification of SSS

18.10.1 This process should occur in the known amplification hood.

18.10.2 Prepare the master mix. Half reactions can be utilized for Fusion 6C amplification. Use the volumes listed on the extraction worksheet (QR-14g or QR-14f) or the amplification worksheet.

18.10.3 Pipette the master mix into the wells or tubes.

18.10.4 Pipette the genomic DNA into the wells or tubes. Be sure to incorporate the amplification positive and negative controls. Use the volumes listed on the extraction worksheet (QR-14g or QR-14f) or the amplification worksheet.

18.10.5 Run the appropriate program on thermal cycler.

18.11 Analysis of database samples

18.11.1 After injecting a set of samples on a 3130 (see SOP 4), 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.

18.11.2 The Analyst shall analyze the data as described in the DNA SOP 31: Fusion 6C. Using analytical peak height thresholds of 50 RFU is acceptable, but samples must be checked for non-artifact peaks above 25 RFU. Routine click-offs do not need to be noted on the analysis worksheets. The positive control(s) (EP1 and

POS) DNA profile(s) must be checked using QR-37 (GeneMarker Concordance Checker). When using a positive extract control besides EP1(i.e. RKO, KJL or TMP), the control may be checked using other means. Allele changes and sizes of peaks out of bin (OB) should be noted in the comment section of the comment chart (QR-14h), as should any low peak height ratios and possible tri-alleles. Change out of bin 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).

18.11.3 For incomplete or unacceptable profiles, determine if the sample needs to be quantified and/or 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)). Disablethese 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.

18.11.4 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.

18.11.5 If peaks ≥ 50rfu arise in the RB or the Neg on database plates, see the CODIS administrator for approval/documentation of the control. If the CODIS administrator does not approve the control it will be necessary to re-amplify or re-extract affected samples. If not already notified, the CODIS Administrator will notify the DNA TL.

18.11.6 If after re-injection, re-amplification, and/or re-extraction, a complete DNA profile has not been generated, the applicator sponge should be used for extraction. 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.

18.11.7 For acceptable profiles on the comment chart (QR-14h), check the box for good profile (if applicable). , Save and export the project to the folder created in step 18.12.1. The SGF file and the plate name should be the same.

18.11.8 The technical reviewer shall import the SGF file into GeneMarker. The technical reviewer will review the data, but not make any changes. Comments or questions are noted and given to the analyst and maintained on the appropriate QR worksheet (i.e. QR-14g, QR-14h). There is no need to save or export this file.

18.11.9 The technical reviewer shall technically review all paperwork associated with analyzed samples. These checks will be noted on QR-14g .

18.11.10 The analyst shall review and/or make changes that the technical reviewer suggested. If changes were made, save and export the file again.

18.12 Importing database samples into CODIS

18.12.1 Move file folder created in step 18.12.1 to U:\Database-nosuspect unit\Convicted offender data\completed folder.

18.12.2 Insert a removable disk into the computer.

18.12.3 Open GenerMarker HID and the project to be imported. Select Application and Export CODIS.

18.12.4 Change Source ORI and Destination ORI to CTCSP3500

18.12.5 Select PowerPlex Fusion 6C as PCR kit

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

18.12.7 Select CMF 3.0 (.xml) file

18.12.8 Select check box of samples to be exported. Ensure controls, ladders, and samples not to be exported are not selected. Specimen Category for each sample should default to "Convicted Offender" in GeneMarker.

18.12.9 Click OK

12.12.10 Save and export the file to the proper folder.

18.12.11 Copy the file to a removable disk.

18.12.12 Browse to the removable disk. Select Export.

Note: **For projects exported with a September date: Open the exported file in Notepad. Use the Find/Replace All function to find SEPT and replace with SEP. Save project.**

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

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

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

18.12.16 Browse for the file created in step 18.13.10

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

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

18.12.19 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.

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

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

18.12.22 Select Import Reports and choose the file that was just created. An SDIS Import Reconciliation Report should appear on the screen.

18.12.23 Ensure that the correct number of QC samples worked properly. Ensure that the number of new database samples being imported is correct. Note on QR-14g .

18.12.24 Print out this report and attach it to QR-14g .

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

18.12.26 File away with appropriate database paperwork.

18.12.27 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.

18.13 Searching for duplicate specimens

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

18.13.2 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.

18.13.3 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.

18.13.4 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 18.15. 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.

18.14 Disposition of duplicate samples in CODIS

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

18.14.2 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.

18.14.3 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.

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

18.15 Casework Known processing pathway

18.15.1 Pick Up: Buccal knowns arrive at the lab on a regular basis. Pick up all the buccal knowns from Evidence Receiving (ER) as needed.

18.15.2 Known Notifications

18.15.2.1 Case Management (CS) picks up the paperwork and adds the information for the knowns into the "Known Processing Logbook" located on the U: drive. Analysts will also add knowns to this logbook as appropriate.

18.15.2.2 Fill out the following information on the "Known Processing Logbook": case #, submission #, name of known, and analyst assigned to the case.

18.15.2.3 Storage in Pending Known Storage: Transfer the known submissions to the proper storage location in LIMS. File away the submissions in the appropriate boxes in the known storage area.

18.15.2.4 Known Processing Log Book: The Known Processing Log Book can be found on the U: drive. All known processing requests (Blood and Buccal samples) are added to that spreadsheet. Indicate if Identifiler Plus and/or YFiler,amplification is necessary, and/or if there is a rush on the case.

18.15.3 Examination of buccal known submissions and itemization in LIMS

18.15.3.1 Upon notification that a known has been submitted to the lab, or when necessary, the assigned analyst or designee for known processing will examine the known(s) and submit the sample(s) (FTA buccal/blood, swab, filter paper cutting, etc.) for extraction.

18.15.3.2 Place one FTA card per suspect/victim in a foil envelope with a desiccant packet. Seal, initial the seal, and label the front of the foil envelope with the case #, item #, and name. In LIMS, itemize the Buccal FTA cards (#X-1, where X = submission #).

18.15.3.3 After examination of the FTA card and prior to transferring to "DNA KnownsTo Be Tested", print out and apply the appropriate barcode to the foil envelope.

18.15.3.4 In LIMS, transfer the buccal FTA cards (in foil envelopes) from yourself to DNA Knowns To Be Tested , unless samples are being processed on the EZ1 right away; in that case they can move forward to processing steps directly.

18.15.3.5 Place the FTA cards in the appropriatebin located in the Known Processing area.

18.15.3.6 The submission packaging will be returned to a secure evidence storage area designated for completed knowns.

18.15.3.7 For buccal known submissions containing only swabs or filter paper saliva samples (or if the FTA card fails to produce a full DNA profile), use around a ¼ portion of one full submitted swab or a cutting of filter paper to obtain a DNA profile. Place the portion to be tested in a tube in a rack in the appropriate bin located in the Knowns Processing area.

18.15.3.8 Retain a portion of the submitted swab(s) (usually the remaining ¾ portion from initial examination) or filter paper, whenever possible, at the laboratory. Transfer the retained swab or filter paper portion physically and virtually in LIMS to Freezer Storage.

18.15.3.9 The testable portions of swabs are typically itemized #X-G1* while the retained portions of swabs are typically itemized #X-G1.

18.15.3.10 If the known sample is coming from a sexual assault kit and no blood is available, select the #X-H (saliva) sample if available. If only #X-E (oral sample) is available, the sample will be treated as a pseudo-known (pending no oral assault) and processed using the EZ1 Advanced XL (See SOP-19 and SOP-20).

18.15.4 <u>Examination of Blood FTA Knowns</u>:

18.15.4.1 The assigned analyst or designee for known processing will examine blood FTA cards, and transfer (from their storage location) virtually and physically to DNA Knowns To Be Tested. If blood samples were previously examined by FB analysts, the evidence transfer sheet may be used in place of the physical evidence sheet to document known processing.

18.15.4.2 Prior to transferring to DNA Knowns To Be Tested, print out and apply the appropriate barcode to the foil envelope (if a barcode is not already located on the envelope).

18.15.5 <u>Batch processing of knowns</u>:

18.15.5.1 FTA cards are punched with a 3mm punch (either by hand or on the punchbot) into tubes or 96 well plates.

18.15.5.2 DNA extraction of FTA cards and swabs follow the standard robotic extraction protocols (see SOP-18.7 through 18.10 (pending number change), SOP-19, and SOP-20).

18.15.5.3 For samples processed with EZI extraction methods, the analyst or designee should transfer the item from DNA Knowns To Be Tested or other applicable location to the analyst's or designee's custody for processing.

18.15.5.4 When sample processing and first analysis by a qualified analyst is completed, the first analyst prints electropherograms to be included in case jackets (for Known samples) and relevant control electropherograms (RB, NEG, EP1, and POS) for the batch and initials all benchwork worksheets (from extraction through injection). (See SOP-31 Fusion 6C)

18.15.5.5 For casework 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.

18.15.5.6 If called peaks arise in the RB or Neg on casework known sample plates, re-prep, re-amplify or re-extract samples as necessary. Samples may be used with TL approval.

18.15.5.7 After analysis is complete, the first analyst may use the electronic cross-comparison checker tool located on the S: drive to confirm no cross contamination, staff contamination, or duplicate sample events occurred within

the batch. If there is a duplicate sample, ensure it is either identical twins or the same sample being processed for different cases, otherwise notify the TL. If the analyst does not use this tool, ensure a manual check of the batch is performed to confirm no contamination event has occurred. Make the appropriate check on QR--300.

18.15.5.8 All paperwork for one batch is transferred to the Technical Reviewer (TR) for data analysis and review of bench paperwork. Please refer to DNA WI-34 for Technical Review process. Please note that for known samples, routine edits made to the electronic data are not needed in the comment fields.

18.15.5.9 The first analyst reviews the packet and makes corrections when necessary. The first analyst separates electropherograms by case, and photocopies the injection worksheets associated with Known sample(s) for each case in the set. All original worksheets (extraction, quant, amp, injection) and control electropherograms for each batch are stapled together and retained in the appropriate folder located in the Known Processing Filing Cabinet or designated area.

18.15.5.10 The electropherogram of the known, the injection sheet, the evidence receipt and physical exam worksheet will be distributed to the analyst assigned to the case for that known.

18.15.5.11 Update the Knowns Log Book on the U-drive. Put samples that failed to give a full DNA profile back on the list for reprocessing.

18.15.5.12 For analyzing casework known samples, the analyst and technical reviewer who analyze and technically review the raw data of the known sample may, or may not be the author and the technical reviewer of the entire case and report. The analyst will initial every electropherogram in the known set analyzed showing acceptance and review of the data. The technical reviewer will document acceptance and review of the data on the proper worksheets.

18.15.5.13 Administrative review will consist of checking for typing errors and check marks. Proper documentation will be made on QR-4A.

18.16 Post Processing Storage of Knowns

18.16.1 Following the generation of a complete DNA profile for each known, transfer samples to the appropriate storage locations.

18.16.2 In LIMS, Buccal FTA cards are transferred to FTA Buccal Known Storage (long-term storage location), and the samples are filed in the appropriate boxes.

18.16.3 In LIMS, Blood FTA cards are transferred to FTA Blood Storage, and the samples are filed back in the appropriate boxes. If blood FTA cards came from the OCME, place the evidence package in a manila envelope, seal it, and note case #, incident town, and "empty OCME packaging" on the envelope. Retain the manila envelope with Trace Storage. In LIMS, the submission is transferred to Trace Storage – retained trace".

18.16.4 In LIMS, Consumed Swabs/cuttings are transferred to Sample Consumed in Testing.

18.16.5 In LIMS, Retained Swabs/cuttings are transferred to Freezer Storage, and the samples are placed in the appropriate bin in the walk-in freezer.

18.16.6 In LIMS, remaining submission packaging and non-tested/retained items are transferred to DNA-Knowns Completed. The samples are placed in the designated storage area.

18.17 <u>Submission packaging return to the submitting agency</u>

18.17.1 It is the responsibility of the designee for known processing to send the submission packaging back to the submitting agencies when a profile has been developed for that known. This can occur before or after the case report has been sent out.

NOTE: See attached Appendix V for flow chart of Casework Known Processing.

APPENDIX I: Punching samples on the BSD Duet:

<u>Turning on the BSD Duet</u>

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. See Sections 18.3 thru 18.6 for assistance.

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 or 3.0mm) 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-14, f, or g. 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.

APPENDIX III: Extraction of SSS on the Biomek 3000.

1. Turn on the Biomek 3000 computer and log on.

2. Make sure nothing is on the deck that can obstruct the robot.

3. Do not lean into the work deck area when robot is on.

4. Make sure your hair is pulled back when using the robot.

5. Turn on the Biomek 3000.

6. Turn on the heat block.

7. Select the Biomek Software icon on the desktop.

8. Select the Instrument pull-down menu, then Home All Axes, and OK. Prompts will ask for confirmation that no liquid is in the tips, no tips or tools are loaded, and identifies the location of the pod (motor head).

9. Select the File pull-down menu, then Open, then B3K_DNAIQV2.0.1.CTDPS, and OK.

10. Select Run.

11. A window prompt will ask for an elution volume. The elution volume should be 40µl.

12. A second window prompt will ask for the End Column.
    a. This number represents the number of rows in which you will be extracting samples.
    b. For example, if extracting a full plate, the end column should be 12.
    c. If extracting samples only in the first row, the end column should be 1.

13. A third window prompt will ask for the First Tip Column.
    a. On the deck, only position ML1 can have a partial box of tips.
    b. All empty rows of tips must be to the left of the first full row of tips.
    c. Full rows of tips must be to the right of the first full row of tips.
    d. There shall be no partial rows of tips.
    e. Once you enter the First Tip Column number, the program will then calculate the number of tips used and will notify you how many more boxes of tips are required for the program to proceed.

Note: A partial box of tips must not contain only 1 row of tips. A minimum of two rows of tips is required for the program to run properly. Therefore, when prompted to input First Tip Column, the input number cannot be 12.

14. On the next three window prompts, select OK. Confirm the gripper and MP200 tool are properly installed and the heat block is turned on and set to 85ºC.

15. The last window prompt instructs you to add specific volumes of reagents to the troughs.

Note: Reagents should never be added to troughs while on the Biomek 3000. To avoid evaporation, add reagents to troughs with no more than approximately 15 minutes of incubation time remaining.

16. Once all the reagents have been added, place the troughs on the deck.

17. Place the plates on the deck and confirm proper setup of the deck.

18. Select OK.

19. The program will proceed to run.

20. Upon completion:
    a. All used tips (found in the waste container) should be discarded. If a partial box of tips is present, cover and place in deck position "ML1". The next extraction on the Biomek 3000 can use those tips as their first partial box.
    b. Discard all working plates.
    c. Clean all troughs
    d. Turn off the heat block.
    e. Turn off the Biomek 3000 robot and computer

21. If the plate contained any buccal swabs, add 160µl sterile water to each well containing DNA extracted from a swab, and also to the appropriate RB, to bring the total volume to 200µl for these samples.

22. Cover your genomic DNA with appropriate film and place in -20°C freezer. Alternatively, proceed to manual amplification of SSS (see section 18.10) or amplification using the Biomek 3000 (see section 18.9).

Appendix IV: Biomek 3000 Maintenance

The Biomek 3000 is a platform that is used to run multiple programs which use multiple pipette heads and a gripper. The complete performance check will take into account the multiple uses of the Biomek 3000. A Performance Check (running programs Performance Check 1 and Performance Check 2 should be run after preventative maintenance, a service call, when the deck has been aligned, or the deck has been completely disassembled (i.e. for a deep cleaning). A complete performance check should be performed at minimum on a yearly basis. This includes testing the Teleshaker and Heat Block. Performance Check 3 should be run after preventative maintenance, a service call, or the deck has been completely disassembled (i.e. for a deep cleaning).

Performance Checks are documented on DNA QR-287. Maintenance is documented on DNA QR-286.

Aligning the deck

1. This should be performed after the robot crashes repeatedly, has been moved and/or disassembled for repair or cleaning.

2. The deck alignment may be done by a trained laboratory employee using the instructions below or by the Beckman Coulter technician using the companies' programs and instructions or the instructions below. If the deck is aligned by a Beckman Coulter technician, it need not be repeated by a laboratory employee.

3. Clear the deck of all labware except the teleshaker.

4. Select the Biomek Software icon on the desktop and home all axes.

5. Select the Instrument pull-down menu and Deck Editor. This will pull up a diagram of the "IQ-Gripper" deck.

6. Each deck position must be aligned separately.

7. For positions ML1, ML2, ML3, ML4, P1, P2, P3, P4, P5, and P6:
   a. Select the deck position.
   b. Select Manual Teach and Next
   c. Attach the framing tool to the head and manually load the tip on the tool: Preferred tip is P20_Barrier.
   d. Select Line tip up against the position target (the center of the bulls-eye on P/N 391910 or P/N 609120).
   e. Align X,Y Axes:
      1. Align the tip with the center of the bulls-eye.
      2. Visualize where the tip is located, and move the circle on the framing wizard to that position.
      3. Select Go.
      4. Repeat until the tip is located above the center of the bulls-eye.
      5. Select Next.
   f. Align Z Axis:
      1. Place a post-it® between the deck and the tip.

2. Move the tip up and/or down until you can feel a slight pull, or drag, when moving the post-it®.
3 Click Finish.
4. Shift ALP to the new coordinates.
5. Select OK.

8. For position P7 (where teleshaker resides):
   a. Keep the teleshaker on the deck and place a 1.2ml deep well plate on it.
   b. Select the deck position.
   c. Select Manual Teach and Next.
   d. Attach the framing tool to the head and manually load the tip on the tool: Preferred tip is P20_Barrier.
   e. Select Line tip up against a Framing Plate.
   f. Align as in steps 7e and 7f, but instead of using the center of the bulls-eye for your alignment point, use the center of well A01, and the top of the plate.

9. Select save to record the new positions of the deck.

Note: When you run a performance check, after aligning the deck, remember to place the shaker and heat block on the deck.

Performance Check 1

1. Select the Biomek Software icon on the desktop.

2. Select the instrument pull-down menu, then Home All Axes.

3. Select the File pull-down menu, then Open and PC1.

4. Select the Run icon.

5. Set the deck up as described within the program.

6. Follow all the prompts within the program.

7. The first steps are to test the tip heights to the deck. Use the "single step" mode when necessary.

8. The Gripper passes if all plates are in the correct positions and the gripper did not need help with movement of the plates.

Starting position of plate	Ending position of plate
P1	P1
P2	P2
P4	P4
P5	P3
P7	P6
P6	P5

9. The MP200 passes if the tips aliquot properly and the tips move to the proper positions.

10. To check aliquot volumes, use a calibrated micro-pipette to confirm the volumes in the plate in position P1:
    a. Row 1 wells 1-8 should contain approximately 50µl (± 5µl);
    b. Row 7 wells 1-8 should contain approximately 10µl (± 2µl);
    c. Row 12 wells 1-8 should contain approximately 110µl (± 11µl).

Performance Check 2

1. Select the File pull-down menu, then Open, and PC2.

2. Set the deck up as described within the program.

3. Follow all the prompts within the program.

4. The first steps are to test the tip heights to the deck. Use the "single step" mode when necessary.

5. The P200L passes if the tips aliquot properly and the tips move to the proper positions.

6. To check aliquot volumes, use a calibrated micro-pipette to confirm volumes in the plate in position B2:
    a. Wells A01, A06, A12 should contain approximately 10µl (± 2µl)
    b. Wells B01, B06, B12 should contain approximately 20µl (± 2µl)
    c. Wells C01, C06, C12 should contain approximately 100µl (± 10µl)
    d. Wells D01, D06, D12 should contain approximately 150µl (± 15µl).

7. The MP20 passes if the tips aliquot properly and the tips move to the proper

positions.

8. To check aliquot volumes, use a calibrated micro-pipette to confirm the volumes in the plate in position B2:
   a. Row 2 wells 1-8 should contain approximately 1µl (± 0.5µl)
   b. Row 7 wells 1-8 should contain approximately 10µl (± 2µl)
   c. Row 11 wells 1-8 should contain approximately 20µl (± 2µl).

Note: If the gripper or any of the pipettes fail, repeat the performance check. If they fail three times, Beckman Coulter should be called to request a technical service call.

Performance Check 3

Performance check 3 will consist of running an extraction positive and negative control through the entire extraction (DNA IQ on the Biomek 3000), amplification (setup manually due to programming constraints), and analysis process. Multiple controls may be run at the same time, although only one positive and negative control need to perform as expected.

Testing the teleshaker (yearly)

1. Turn on the Biomek 3000 computer (log on) then turn on the robot.

2. Select the Biomek Software icon on the desktop.

3. Select the Instrument pull-down menu, then Home All Axes, and OK. Prompts will ask for confirmation that no liquid is in the tips, no tips or tools are loaded, and identifies the location of the pod (motor head).

4. Select the File pull-down menu, then Open, then Teleshaker PC, and OK.

5. Select the Run icon.

6. The Teleshaker will move for approximately 10 seconds then stop on its own.

7. Visually check the shaker to see if it is moving in an N,W,S,E.

8. Shaker passes if it can shake then stop on its own. If the shaker fails, consult with the manufacturer's instructions.

<u>Testing the heat block</u> (yearly)

1. The heat block is placed in location P6 on the Biomek 3000.

2. Place a 1.2ml deep well plate on the heat block.

3. Fill wells C05, C06, C07, D05, D06, D07, E05, E06, and E07 with water.

4. Place a NIST traceable thermometer in well D06.

5. Turn on the heat block and let sit until stable (10-30 minutes).

6. The digital reading of the heat block can vary between 80-95ºC.

7. To pass, the thermometer in the plate must read 55ºC or higher (up to 85ºC). DNA IQ chemistry requires 50ºC to work properly. If the heat block fails, consult with the manufacturer's instructions for adjusting the temperature of the block.

Note: Please document Maintenance and Performance Checks using DNA QR-286 (Biomek 3000 Maintenance) and DNA QR-287 (Biomek 3000 Performance Check).

SOP-18 Appendix V: KNOWN PROCESSING PATHWAY