

DNA SOP-12 Processing of Casework Knowns / Single Source Samples

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12.1 PURPOSE

Training new personnel and assisting current personnel in processing Casework knowns/single source samples (SSS), comprehensively from setting up worksheets and plates/tubes to extracting and amplifying said samples.

12.2 RESPONSIBILITY

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

12.4 Casework Known processing pathway

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

12.4.2 Known Notifications

12.4.2.1 Case Management (CS) picks up the paperwork and adds the information for the knowns into the "Known Processing Logbook" located on the U: drive. Also, a DNA analyst may add requested knowns to the spreadsheet.

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

12.4.2.3 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.

12.4.2.4 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 Yfiler amplification is-necessary, and/or if there is a rush on the case.

12.4.3 Examination of buccal known submissions and itemization in LIMS

12.4.3.1 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.

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- 12.4.3.2 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 #).
- 12.4.3.3 After examination of the FTA card and prior to transferring to “DNA Knowns - To Be Tested”, print out and apply the appropriate barcode to the foil envelope.
- 12.4.3.4 In LIMS, transfer the buccal FTA cards (in foil envelopes) from yourself to DNA Knowns- To Be Tested, unless samples are being processed right away; in that case they can move forward to processing steps directly.
- 12.4.3.5 Place the FTA cards in the “DNA Knowns- To Be Tested” bin that is in the Known Processing area.
- 12.4.3.6 The submission packaging will be returned to a secure evidence storage area designated for completed knowns.
- 12.4.3.7 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 a portion of the submitted swab(s) or cutting of filter paper to obtain a DNA profile. Place the portion to be tested in a tube in a rack in the “DNA Knowns- To Be Tested” bin.
- 12.4.3.8 Retain a portion of the submitted swab(s) or filter paper at the laboratory. Transfer the retained swab or filter paper portion physically and virtually in LIMS to Freezer Storage.
- 12.4.3.9 The testable portions of swabs are typically itemized #X-G1* while the retained portions of swabs are typically itemized #X-G1.
- 12.4.3.10 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 known (pending no oral assault) and processed using the EZ1 Advanced XL (See SOP-19, SOP-20, and SOP-26).
- 12.4.4 Examination of Blood FTA Knowns:**
- 12.4.4.1 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.

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- 12.4.4.2 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).

12.5 Batch processing of knowns:

12.5.1 Known casework plate sample list preparation

- 12.5.1.1 Buccal FTA samples, blood FTA samples, and buccal swab samples may all be amplified on a single 96-well PCR plate. Swabs must be manually entered onto the amplification worksheet (QR-29).
- 12.5.1.2 If only buccal swab (not FTA) samples, and/or manually punched FTA samples are being extracted, proceed to step 12.5.2: Plate workbook setup. The remainder of this procedure, starting at step 12.5.1.4, refers to FTA blood and buccal samples being punched using the BSD Duet.
- 12.5.1.3 In LIMS, transfer all the samples to be tested to “DNA-Knowns– To Be Tested”. (Prior to transferring, this location should be empty.) A maximum of 84 samples can be tested.
- 12.5.1.4 Select the Crystal Reports icon.
- 12.5.1.5 Select the DNA Casework Known Plate Sample List, then Print, and Screen.
- 12.5.1.6 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 on a removable disk or shared drive.
- 12.5.1.7 Open the Direct Amplification Worksheet (QR-29). In the LIMS tab, click “Import Sample List” to import the previously saved crystal report in step 12.5.1.6. 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.
- 12.5.1.8 After the macro is run, manually type in any swab samples, and/or manually punched FTA samples not being punched robotically to QR-29.

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12.5.1.9 If any casework known buccal swabs (not FTA cards) or manually punched FTA cards are to be amplified, fill their names into appropriate wells. A separate RB must be added to the plate for the buccal swab samples.

12.5.1.10 In LIMS, transfer all samples from “DNA-Knowns – To Be Tested” into your name.

12.5.2 Plate workbook setup

12.5.2.1 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 worksheet: QR-29.

12.5.3 Punching a plate using the BSD Duet

NOTE: Detailed instructions can be found in Appendix I of SOP-28 and QR-29.

12.5.3.1 Turn on the BSD Duet.

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

12.5.3.3 Use the barcode reader when applicable.

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

12.5.3.5 Turn off the BSD Duet when done.

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

12.5.3.7 This method is used for samples on 96-well plates for Fusion 6C Direct Amplification.

12.5.4 Manual Punching of FTA Cards

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

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

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12.5.4.3 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).

12.5.4.4 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.

12.5.5 Amplification of Single Source Samples Using Fusion 6C Direct

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

12.6 Analysis of Known Samples (overview)

Note: See SOP 5 (Identifiler) and SOP 26 (Fusion 6C Direct) or SOP 30 (Fusion 6C)

12.6.1 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-5: PCR Interpretation of Results (Identifiler Plus) and SOP 27 (Fusion 6C Direct PCR Interpretation).

12.6.2 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.

12.6.3 **For Identifiler Plus only:** If * peaks or called peaks arise in the RB or Neg on casework known sample plates, re-amplify or re-extract samples as necessary. Samples may be used with TL approval.

12.6.4 **For Fusion 6C (Direct or regular):** If called peaks arise in the RB or Neg on casework known sample plates, re-amplify or re-extract samples as necessary. Samples may be used with TL approval.

12.6.5 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, or duplicate

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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 the corresponding QR worksheet.

- 12.6.6 All paperwork for one batch is transferred to the Technical Reviewer (TR) for data analysis and review of bench paperwork. The TR reviews all bench paperwork and reviews the packet to ensure each page is initialed by the first analyst. The TR forwards all paperwork for administrative review, then returns it to the first analyst.
- 12.6.7 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.
- 12.6.8 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.
- 12.6.9 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.
- 12.6.10 For analyzing casework known samples, the 1st and 2nd analysts who analyzed 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 1st analyst will initial every electropherogram in the known set analyzed showing acceptance and review of the data. The 2nd analyst will document acceptance and review of the data on the proper worksheets.
- 12.6.11 Administrative review will consist of checking for typing errors and check marks. Proper documentation will be made on QR-4A.

12.7 Post Processing Storage of Knowns

- 12.7.1 Following the generation of a complete DNA profile for each known, transfer samples to the appropriate storage locations.
- 12.7.2 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.

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- 12.7.3 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”.
- 12.7.4 In LIMS, Consumed Swabs/cuttings are transferred to Sample Consumed in Testing.
- 12.7.5 In LIMS, Retained Swabs/cuttings are transferred to Freezer Storage, and the samples are placed in the appropriate bin in the walk-in freezer.
- 12.7.6 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.
- 12.8 Submission packaging return to the submitting agency**
- 12.8.1 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 for flow chart of Casework Known Processing.

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SOP-12 Appendix: KNOWN PROCESSING PATHWAY

