1. Scope

This protocol describes the general approach to Forensic Biology examinations. The protocol is not meant to encompass all possible circumstances that may be encountered in forensic casework. An analyst may deviate from this protocol as circumstances necessitate based on his/her training and experience. Significant deviations must be approved by the DNA Technical Leader.

2. References

2.1. Ensuring high standards, in: DNA Technology in Forensic Science, National Academy Press, Washington, D.C., 1992, pp. 97-110.
2.2. Ensuring high standards of laboratory performance, in: The Evaluation of Forensic DNA Evidence, National Academy Press, Washington, D.C., 1996, pp. 75-88.
2.3. DNA Advisory Board, Quality assurance standards for forensic DNA testing laboratories, 2000.
2.4. R. Saferstein, Examining evidence and removing biological stains, in: Forensic Science Handbook, Vol. I, second ed., Prentice Hall, Upper Saddle River, 2002, pp. 527-530.
2.5. N. Vandenberg, R.A. van Oorschot, The use of Polilight® in the detection of seminal fluid, saliva, and bloodstains and comparison with conventional chemical-based screening tests, J. Forensic Sci. 51 (2006) 361-370.
2.6. J.M. Butler, Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers, second ed., Elsevier Academic Press, Burlington, 2005.

3. Equipment

3.1. See equipment for appropriate protocols.

4. Safety/Quality Assurance

4.1. See Safety section for appropriate protocols.

5. Procedure

5.1. General Contamination Prevention Practices

5.1.1. At a minimum, a disposable lab coat, disposable gloves, and eye protection will be worn while examining evidence or performing procedures in the DNA laboratory. A face mask may be worn at the analyst's discretion.

5.1.1.1. All visitors must wear a disposable lab coat, face mask, and disposable gloves, unless those individuals choose to provide a DNA sample for the CODIS Staff Index.

5.1.2. Prior to and/or after examination of evidence, DNA extraction, PCR setup, and post-amplification procedures, examination work areas (hoods, countertops, etc.) and tools (tweezers, scissors, etc.) will be cleaned with a 10% bleach solution followed by 70% ethanol. Centrifuges, pipettors, Alternate Light Sources, and other equipment will be cleaned with 70% ethanol prior to and/or after use. Additionally, all evidence will be examined on a new sheet of bench paper.

NOTE: If examination of evidence takes place in the laboratory of a different section (Explosives, Latent Prints, etc.), these cleaning methods may not be feasible. In such cases, the cleaning methods/evidence handling practices of that section would apply.

5.1.3. All tube racks will be cleaned in a 10% bleach bath followed by rinsing with water after use. The 10% bleach bath will be made every two weeks.

5.1.4. At a minimum, all of the surfaces, hoods, pipettors, centrifuges, and other equipment in the DNA laboratories will be cleaned on a quarterly basis with the appropriate cleaning solutions (10% bleach solution followed by 70% ethanol or 70% ethanol only). The **quarterly** cleanings shall be documented in the appropriate log.

5.1.5. UV Crosslinkers will be used on the following items, at a minimum:

5.1.5.1. Tube racks, canisters – prior to use and after each cleaning

5.1.5.2. Non PCR-ready microcentrifuge tubes, 0.2 mL microcentrifuge tubes, and DNA IQ™ Spin Baskets

5.1.5.3. Aliquots of nuclease-free water and TE^{-4} prepared in 50 mL conical tubes (TE^{-4} purchased pre-mixed does not need to be cross-linked)

5.1.6. Contamination will be monitored by the evaluation of the control samples routinely analyzed during the course of casework analysis (i.e. extraction reagent blanks, positive controls, amplification negative controls, and quantitation plate blanks).

5.1.6.1. Contamination noted in negative controls (reagent blanks and amplification blanks), positive controls, or evidence samples that is observed during analysis and corrected by setting up a new plate for the CE does not require entry into the contamination log, a *Correction*, or *Corrective Action.* If the contamination occurred prior to CE analysis (e.g. DNA extraction, amplification, etc.), the event will be documented in the contamination log. If the contamination event appears to be indicative of a pattern or systemic problem, a *Correction* or *Corrective Action* may be initiated.

5.1.7. A contamination log will be maintained containing contamination incidents and the related documentation.

5.1.8. At a minimum, the contamination log book shall contain the following:

5.1.8.1. Case number

5.1.8.2. Analyst

5.1.8.3. Date

5.1.8.4. Sample contaminated (e.g. reagent blank, positive control, etc.)

5.1.8.5. Brief description of the incident including when the contamination was detected, investigation as to the source of the contamination, and the resolution.

5.1.8.6. Contaminating DNA profile

5.1.8.7. Documents such as case worksheets, notes, and electropherograms considered relevant to the investigation and resolution of the contamination event.

5.1.9. Staff Hits: If a DNA profile developed from evidence matches a member of the laboratory staff:

5.1.9.1. The contamination event will be documented in the contamination log (see above).

5.1.9.2. If the staff contamination is a single event and does not appear to be indicative of a pattern or systemic problem, a *Correction* may be documented.

5.1.9.3. If the staff contamination appears to be indicative of a pattern or a systemic problem, then a *Corrective Action* shall be initiated.

5.2. Sample Contamination Prevention Practices

5.2.1. To avoid sample mix-up or contamination, work with only one item at a time.

5.2.2. Handle evidence samples believed to contain low levels of biological material before samples believed to contain higher levels.

5.2.3. In general, evidence samples will be processed prior to the examination of reference samples. Evidence samples and reference samples will undergo evidence processing, extraction, quantification setup, and PCR setup in separate designated areas.

5.2.4. Pulse spin all microcentrifuge tubes before opening to remove liquid from the inside of the cap. Use care when opening microcentrifuge tubes to prevent splashing.

5.2.5. Only relevant paperwork will be carried into and out of the post-amplification laboratory. Do not return this paperwork to the pre-amplification laboratories.

5.2.6. Hands shall be washed prior to leaving the post-amplifcation laboratory.

5.2.7. To prevent sample switching:

5.2.7.1. Immediately prior to making a sample transfer, verify the labels on the sample tube and the new tube.

5.2.7.2. After making a sample transfer, it is recommended to move the sample tube(s) to a different location within the rack, or a different rack, to prevent using the same tube twice.

5.3. General Evidence Examination Procedure

5.3.1. Items will first be visually examined to determine whether a Forensic Biology exam is appropriate. In those instances where a Forensic Biology exam is not appropriate, the reason(s) will be recorded in the analyst's notes.

5.3.2. An Alternate Light Source (ALS) may be used by the analyst to assist in conducting the visual exam. It may be useful when examining clothing for biological stains or to help locate areas of the clothing to sample when attempting to determine the wearer. Refer to the Alternate Light Source protocol for proper use of the ALS.

5.3.3. If hairs are observed on an item, or if a Trace Evidence exam has been requested, the analyst will take precautions to assure that no hairs or other trace evidence is lost during the Forensic Biology exam. A Trace Evidence examiner shall be consulted before proceeding with DNA analysis on hair roots.

5.3.4. After a visual examination, applicable serology examinations may be conducted at the analyst's discretion based on the circumstances (sample size, probative value, etc.).

5.3.5. The phenolphthalein test can be used as a presumptive test for blood. Refer to the Phenolphthalein Test for Blood protocol for detailed instructions on performing this procedure.

5.3.6. The analyst may swab, take cuttings, or use another approved method to collect biological evidence from items for PCR testing. The size of cuttings and/or number of

swabs used will be determined by the analyst based on his/her training and experience. The analyst should try, if feasible, to leave a portion of the stain untested in the event that re-testing is required. If this is not feasible, the analyst will obtain permission from the prosecuting attorney or investigating agent to consume the stain. The approximate size/amount of stain cut for DNA and/or remaining should be indicated in the analyst's notes.

5.3.7. DNA extraction and purification of evidence samples will be performed at a separate time/space from reference samples. Refer to the appropriate extraction protocol for detailed instructions on performing this procedure. In general, semen-containing samples will not be analyzed by the ATF Laboratory and should be forwarded to a laboratory that routinely analyzes this sample type.

5.3.8. Multiple swabs/cuttings from the same stain/item may be extracted in separate tubes and then combined into a single sample using a Microcon filter.

5.3.9. The DNA concentration of the extracts will be estimated using the Quantifiler® Kit and the Applied Biosystems™ 7500 Real-Time PCR instrument. Refer to the Quantifiler® protocol for detailed instructions on performing this procedure.

5.3.10. If necessary, samples may be concentrated using Microcon® filters. Refer to the 30 kD Microcon® DNA Concentration/Purification protocol for detailed instructions on performing this procedure.

5.3.11. Using the quantification data, samples will be amplified using the GlobalFiler™ PCR Amplification Kit and a validated thermal cycler. The target concentration of DNA template for this amplification will generally be 0.5 ng. It may be necessary for the analyst to amplify a lesser or greater amount of DNA depending on the sample type/situation. The analyst will use his/her training and experience to make the determination on when this should be done. Refer to the GlobalFiler™ protocol for detailed instructions on performing this procedure.

5.3.12. DNA typing will be performed on all amplified samples using a validated Applied Biosystems™ Genetic Analyzer. Samples may be injected multiple times at the analyst's discretion. Refer to the Applied Biosystems™ Genetic Analyzer protocol for detailed instructions on performing this procedure.

5.3.13. Evidence samples and reference samples from the same case may not be loaded on the same Genetic Analyzer plate.

5.4. Disposition of Evidence

5.4.1. When a case has been completed, the associated evidence (exhibits and DNA extract tubes) will be properly sealed and returned to the evidence custodian. If the entire extract has been consumed during analysis, the extract tube may be discarded. The remaining extract tubes shall be packaged individually (e.g. small zip-lock bags). All extracts associated with the case (multiple submissions, if desired) will be sealed in an outer envelope labeled at a minimum with the case number, exhibit number(s), analyst's initials, and date. The extracts will be retained by the ATF Laboratory at approximately -20°C. The disposition of the extract tubes (i.e. retained or discarded) will be recorded in the closing inventory. Amplified product shall be considered work product and discarded after the case report has undergone a technical review.

5.4.2. Prior to returning the original evidence, the outer packaging of all exhibits submitted to the laboratory for DNA analysis that may reasonably still contain biological material will be labeled so as to clearly identify the exhibit as potentially containing biological evidence and therefore should be stored in a temperature controlled environment.