DNA Procedures Introduction

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1 INTRODUCTION

Quality assurance measures ensure that laboratory tests yield results that accurately reflect the physical parameters they seek to characterize. As a part of the Laboratory Division quality program, the DNA Units, which include the DNA Casework Unit (DCU), DNA Support Unit (DSU), Federal DNA Database Unit (FDDU) and Scientific and Biometrics Analysis Unit DNA Group (SBAU), have developed technical procedures, or standard operating procedures (SOPs), for the various methods used to perform laboratory activities. Each biology discipline SOP specifies the pertinent materials needed and the procedural steps to perform the test or tasks associated with the method to ensure the uniformity of each technique's performance over time and across scientists. This document supplements each technical procedure and provides guidance for the proper preparation of laboratory equipment and space, use of personal protective equipment (PPE), and general laboratory guidance.

2 SCOPE

This document applies to DNA personnel that work on forensic evidence samples, casework reference samples, and/or DNA database samples.

3 GENERAL PRECAUTIONS AND CLEANING

3.1 Pre-Amplification vs Post-Amplification

- Pre-amplification work areas are separated from post-amplification work areas.
- Amplified DNA is stored in the post-amplification work areas and must not be moved into the pre-amplification work areas.
- Examination of evidence or sample accessioning, DNA extraction, and amplification setup procedures may be performed in the same pre-amplification laboratory rooms if performed at separate times or at separate workstations.

3.2 Work Surfaces

- All work surfaces in pre-amplification laboratory space must be decontaminated with a 10% bleach solution each workday before use, as they become visibly soiled, and after their final use on a given workday.
- All work surfaces within the post-amplification laboratory must be cleaned weekly, generally with a detergent (e.g., CaviCide, multipurpose cleaner) and water.
    - Using bleach on the capillary electrophoresis instruments may interfere with fluorescence and should be avoided.
- Disposable paper (e.g., bench paper, weigh paper, tissue paper) is used when processing evidence items to ensure a clean working surface and to prevent the deposition of biological material on permanent work surfaces.
    - Disposable paper must be changed and appropriately discarded as it becomes visibly soiled or, at a minimum, before and after the completion of the examination of an individual item of evidence.

- Evidence items that are packaged together (e.g., vaginal swabs, clothing items) may be processed on the same disposable paper, provided that the paper does not display visible soiling.

3.3 Extraction Work Areas

- DNA extraction procedures begin with the addition of the extraction reagents to the sample collected for DNA typing.
- All manual DNA extraction procedures must be conducted within a hood unless otherwise indicated.
- DNA extraction steps in which phenol/chloroform/isoamyl alcohol (PCIA) reagent is used must be performed in a chemical fume hood.
- Manual DNA extraction and amplification setup procedures must be conducted within separate hoods or at separate times if performed in a common hood.
- Automated DNA extraction and amplification setup procedures must be conducted on separate Robotic Workstations or at separate times if performed on a common Robotic Workstation.

3.4 Personal Protective Equipment (PPE)

In addition to safety (see section 8), PPE is utilized to minimize the potential for introduction of biological material by Laboratory personnel and prevent DNA contamination.

3.4.1 Gloves

- Disposable gloves must be used at all times during examination of evidence and sample processing.
- At a minimum, gloves must be changed if they become visibly soiled, torn, or when moving between separately packaged evidence items, with the exception of Sexual Assault Kit (SAK) swabs.
    - Personnel are not required to change gloves between swabs collected from a single individual within a SAK.
- To prevent transfer of biological material to laboratory surfaces that are not easily decontaminated (e.g., telephones, computer keyboards), used gloves should be removed prior to handling such laboratory devices.
    - Double gloves may be worn to facilitate the removing and donning of outer gloves.
- Gloves must be changed and/or surfaces should be cleaned if inadvertent contact with a surface that may result in transfer of biological material is suspected (e.g., answering phone, scratching face).
- When handling evidence items with potential latent fingerprint value, cloth gloves may be worn under the disposable gloves during processing.
    - Nitrile gloves are preferred when processing items of potential latent fingerprint value.
- Prior to leaving the laboratory area, used gloves must be properly discarded and personnel should wash their hands.

3.4.2 Lab Coats

- A laboratory coat must be worn during all pre-amplification processes.
- A separate laboratory coat should be worn during post-amplification processes.
    - Laboratory coats that are used in post-amplification laboratory space must not be worn into pre-amplification laboratory space.
- Laboratory coats should be placed in a laundry receptacle upon becoming visibly soiled.
- Laboratory coats must not be worn outside of designated laboratory space unless transporting evidence or samples.

3.4.3 Face Masks

- Disposable face masks must be used at all times when handling evidentiary items or database samples, and when performing pre-amplification processes.
- Face masks must also be worn when preparing pre-amplification reagents.
- Face masks do not need to be worn during post-PCR amplification processes.
- At a minimum, face masks must be changed if they become visibly soiled or torn.

3.4.4 Eye protection

- Eye protection should be worn when performing laboratory testing and when preparing reagents or chemicals outside of a hood.

4 EQUIPMENT USE AND CLEANING

4.1 Pre-Amplification vs Post-Amplification Equipment

- Supplies and equipment will be dedicated for pre- or post-amplification work areas and will not be moved from post- to pre-amplification work areas unless decontaminated.
- Post-amplification supplies and equipment will not be stored in rooms used for evidence examination or database sample processing unless decontaminated.

4.2 Pipettes

- Handheld pipettes dedicated to pre-amplification work areas must be used when performing pre-amplification activities (e.g., serological examination, sample collection, DNA extraction, qPCR set-up, amplification set-up) and any other pre-amplification methodologies.
    - Pipettes dedicated to pre-amplification activities must be irradiated using the interior ultraviolet (UV) light of the biological hood, a stratalinker, or equivalent for at least 5 minutes each workday before use. Pipettes should also be UVed after their final use on a given workday.
    - Pipettes must be thoroughly decontaminated with a 10% bleach solution and then disinfected/rinsed with 70% isopropyl alcohol each workday before use, as they become visibly soiled, and after their final use on a given workday.

- A different dedicated set of handheld pipettes must be used when performing post-amplification activities (i.e., sequencing, capillary electrophoresis set-up) and transferring liquid that potentially contains amplified DNA.
    - Pipettes dedicated to post-amplification activities must be cleaned weekly and as they become visibly soiled.
- Sterile disposable pipette tips or transfer pipettes must be used when transferring liquid reagents or samples.
    - A new pipette tip or transfer pipette must be used when removing sample from a tube or when introducing reagent into a tube that contains sample.
    - The tip or transfer pipette must be discarded in the appropriate waste container after use.
- To minimize the potential for pipetting inaccuracies, a pipette with a range larger than and closest to the target volume should be used.
    - The pipette should be set to the desired volume by initially dialing into the range of volumes larger than the target volume and then dialing back to the desired volume.

4.2.1 Robotic Workstations

- Robotic workstations that use fixed tips must be appropriately flushed with bleach and/or water between each sample and at the conclusion of a procedure.

4.3 Tools

- New or clean forceps, scalpel blades, or scissors must be used for every sample.
    - Tools must be appropriately discarded or decontaminated with a 10% bleach solution followed by 70% isopropyl alcohol between consecutive samples.
    - Additionally, tools used for mitochondrial DNA (mtDNA) evidence examinations may be exposed to UV light before use.

4.4 Hoods

- In addition to bleaching the worksurface (see section 3.2), biological hoods must be irradiated with their interior UV light for at least 5 minutes each workday before first use and after final use.
- A 15-minute exposure time is recommended for mtDNA processes.

4.5 Performance Verification

- For equipment that requires a performance verification (i.e., performance check or quality control checks) prior to use, the results will be recorded in accordance with LAB-100 and the applicable DNA quality assurance and/or quality control procedures.

5 REAGENTS

- Reagents are stored separately from evidentiary and database samples. If the same storage area is used, at minimum, reagents must be placed on a different shelf and above evidentiary material or database samples.
- Casework reagents will generally be dispensed into small aliquots to minimize the number of times the stock reagent is opened.
- When necessary, reagent tubes should be thawed completely and vortexed briefly before use. As appropriate, the tubes can be quick spun (approximately 2 seconds) to return all liquid to the bottom of the tube.

6 EVIDENCE AND SAMPLE HANDLING

- Refer to LAB-200 and BIO-201 for additional requirements pertaining to evidence, evidence handling, and evidence management.
- DNA personnel should use the amount of evidence considered necessary to provide DNA typing results. Refer to BIO-101 for additional requirements pertaining to consumption.
- All evidence items or database samples under examination must be kept separate from other items of evidence or database samples under examination by any other individual(s) working within a common laboratory space.

6.1 Sample Tube Handling

- While manually processing samples through a common procedural step, only one sample tube or reagent tube should be open at a time. Remaining sample or reagent tubes should remain closed.
- All sample containing tubes that do not display a visible difference after the completion of a procedural step (e.g., color change, volume change, cutting introduction) must be physically moved or marked in a manner that distinguishes them from those on which that step has yet to be completed.
    - This requirement will help to prevent the misloading or double-loading of samples during any procedural step that does not result in an evident physical change to a handled sample.

6.2 Sample Storage

- Extract tubes may be stored refrigerated or frozen.
    - Upon retrieval from storage for subsequent examinations, extracted samples should be brought to room temperature, vortexed (approximately 2 seconds), and quick spun (approximately 2 seconds).
- For plate-based samples, before removing an adhered cover (e.g., heat sealed cover) the plate should be centrifuged (approximately 30 seconds) to return all the liquid to the bottom of the wells.
- Upon completion of testing, extract tubes may be stored at room temperature if the remaining extract is dried down. Instructions for using the Speed-Vac or Vacufuge are contained within the applicable technical procedure (i.e., BIO-520).

7 NOTES AND TECHNICAL RECORDS

- During the examination of an evidence item, notes (e.g., description of item, test result) must be recorded contemporaneously with conducting a procedure on or sample collection from that item.
- Such notes must be recorded in their final form (i.e., entered electronically). When contemporaneous notes must be recorded as a physical record, the content may be transcribed into an electronic system provided the original record or a scanned copy is retained in accordance with FBI Information Management Division policies.
- Multiple swabs from the same collection site and packaged together (e.g., vaginal swabs, oral swabs) may be processed together before being individually described in the final case records.

8 SAFETY

- All evidence containing or contaminated with blood or other potentially infectious materials will be considered infectious regardless of the perceived status of the source individual or the age of the material.
- Refer to the FBI Laboratory Safety Manual for information on personal protection, the proper disposal of the chemicals used in these procedures, as well as the biohazardous wastes generated.
- The following safety warnings are noted. Additional safety information is contained in each applicable technical procedure.
    - Direct UV light can be harmful to eyes. UV-protective eyewear should be worn when directly observing UV lights.

8.1 Disposal

- Masks, gloves, bench paper, or tubes that are visibly soiled with biological material (e.g., blood, semen) must be placed into biological waste containers for disposal.
- Disposable items that do not show any visible biological staining may be discarded into regular waste containers.

9 REVISION HISTORY

Revision	Issued	Changes
00	08/01/2022	Reformatted and reorganized DNA 600-2 into new template and assigned new Doc ID. Modifications to wording throughout to update and mirror LAB-100 terminology.
01	04/01/2024	Added guidance to UV pipettes at the end of the day. Updated reference for Evidence Management content moved from BIO-101 to BIO-201. Added reference to BIO-101 for consumption guidance.