Purchasing, Preparation, and Records for DNA Reagents

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1 INTRODUCTION

This procedure supplements the FBI Laboratory requirements for the purchasing and receipt, preparation, and labeling of laboratory reagents and consumables used in the DNA discipline.

2 SCOPE

This document applies to DNA personnel responsible for the purchasing and receipt, preparation, and labeling of laboratory reagents and consumables used for the serological and/or DNA analysis of forensic evidence and/or databasing samples in the DNA discipline.

3 PROCEDURES

A. The DNA units comply with the FBI Laboratory level 1 documents (i.e., LAB-100 and LAB-200) and the *Quality Assurance Standards* (QAS) *for Forensic DNA Testing Laboratories* and *for DNA Databasing Laboratories* with regard to the quality control (QC) of reagents.
B. Reagent records will be maintained, generally via the DNA units' applicable Sample Tracking and Control Software (STACS).
C. Refer to DNA Procedures Introduction (i.e., BIO-100) for additional laboratory quality assurance, cleaning, and reagent storage instructions.

3.1 Commercial Reagent Records

A. Purchase requests will be prepared by appropriate personnel for all commercial reagents and consumables. These requests will describe the types of supplies and/or services requested and may be maintained in a written or electronic format. The appropriate manager will approve purchase requests prior to ordering.
B. Various companies may supply one or more chemicals, reagents, or DNA analysis kits used in the testing of forensic evidence and/or database samples. Final selection of suppliers will be in accordance with Federal Procurement Regulations - Simplified Acquisition Procedures.
    1. Suppliers of critical reagents are typically evaluated during validation but the evaluation may be based on previous purchasing history and/or the results of QC testing.
    2. When a vendor, reagent specification (e.g., concentration), or consumable specification (e.g., Vivicon filter molecular weight cut-off) affects the laboratory activities, the pertinent information will be listed in the appropriate DNA procedure.
    3. Current supplier and purchasing information for reagents and consumables is generally maintained in STACS.
C. DNA personnel will ensure that quality affecting supplies, reagents, and consumables comply with specifications defined in the appropriate technical procedure and/or the purchase request. Any discrepancies will be brought to the attention of the personnel responsible for ordering DNA supplies, reagents, and

consumables. Quality affecting supplies, reagents, and consumables that conform to the expected specifications will be received into STACS.

D. The following information will be recorded for the receipt of commercial reagents and kits:
    - Name of material
    - Manufacturer lot number
    - Date received
    - Expiration date, when provided

E. Commercial reagents must be labeled with the identity of the reagent and will be directly labeled and/or use a barcode system to electronically track the expiration date.
    1. Commercial reagents are generally prepared and stored as recommended by the manufacturer. Additional guidance may be found in STACS or the appropriate DNA procedure.
    2. The expiration date of commercial reagents is determined by the manufacturer or utilizing the respective Safety Data Sheets.
        i. If no expiration date is provided by the manufacturer, generally a 10-year expiration date will be assigned.
        ii. If the expiration date provided by the manufacturer only specifies a month and year, the recorded expiration date will be the last day of the month specified. The commercial reagent container will then be labeled with the newly established expiration date.
        iii. If an expiration date beyond that provided by the manufacturer is assigned, records to establish the extension of the expiration date will be maintained.
        iv. If an expiration date is exceeded, the QC procedure or use of the reagent on a known sample (e.g., positive control) may be used to demonstrate continued efficacy beyond the assigned expiration date. Records demonstrating the continued efficacy of the reagent will be maintained.

F. If a non-reagent consumable (e.g., swabs, tubes, microcons, centristrips) has an expiration date assigned by the manufacturer, that expiration date is not applicable to DNA units' operations and may be extended as deemed appropriate by the Technical Leader (TL). The new expiration date will be recorded in STACS.

G. Water must be of suitable purity so that it does not interfere with the specificity, accuracy, and precision of the procedure.
    1. For water that will contact DNA samples or be used to make reagents for DNA testing, the water should be nuclease free. This is typically referred to as reagent grade, molecular grade, or nuclease free in the procedures and notes.
    2. The purified water available via faucets at the laboratory sinks is used for Tecan operation or general laboratory uses that do not involve processing of samples for DNA.

3.2 Laboratory Prepared Reagent Records

A. Laboratory prepared reagents will be made in accordance with the information contained within STACS and/or the relevant DNA procedure(s). A list of reagent recipes and control preparation guidelines generally used in the DNA units are listed in Appendix A for reference.

B. Reagent preparation will be recorded, generally within STACS, to include:
   - Name of reagent
   - Date prepared
   - Lot number (e.g., barcode, batch identifier) assigned to the reagent
   - Lot number (e.g., barcode, batch identifier) of each component
   - Identity of the preparer
   - Identity of the individual performing the QC check (if applicable)
   - Lot/batch # of the QC controls (if applicable)
   - QC results (pass/fail) (if applicable)

   1. The information recorded for reagent preparation and use must be sufficient to provide a documented audit trail.
   2. Unless otherwise specified, reagents that are made internally will expire one year from the date prepared. If an expiration date is extended, records demonstrating the continued efficacy of the reagent will be maintained.
      i. If an expiration date is exceeded, the use of the reagent on a known sample may be used to demonstrate continued efficacy beyond the assigned expiration date. Records demonstrating the continued efficacy of the reagent will be maintained.

C. Laboratory prepared reagents will be labeled with the identity of the reagent and will be directly labeled and/or use a barcode system to electronically track the following:
   - Preparer's identity
   - Date prepared
   - Expiration date
   - Lot number (e.g., barcode, batch identifier)
      i. Reagents stored in limited use quantities (i.e., single use aliquots) may be in a storage container (e.g., bag, box) labeled with the identity of the reagent with the associated barcode(s) readily available.
      ii. Once dispensed for use (e.g., on a robotic workstation, on another instrument), the trough or tube will be labeled with the identity of the contained reagent with the necessary barcode available for scanning.

D. Laboratory prepared reagents will be stored at an appropriate temperature to prevent degradation or deactivation of the active ingredients. Recommended storage conditions may be listed in STACS and/or Appendix A.

E. Laboratory prepared reagents will be tested for reliability prior to or concurrent with use in casework examinations or DNA databasing. QC procedures for reliability testing may be contained in the appropriate DNA procedure or in STACS or reliability

testing may be accomplished by testing appropriate positive and/or negative controls. Dilutions of a stock commercial reagent (e.g., 1X CE buffer) will not require reliability testing. Multiple reagents may be simultaneously tested for reliability.

3.3 Critical Reagents

A. The following are identified as QAS critical reagents and will be evaluated prior to use in casework or for database sample processing:
   - Nuclear DNA Quantification Kits (i.e., Quantifiler TRIO)
   - Mitochondrial DNA Quantitative PCR (qPCR) system:
     - Double stranded synthetic standard (dsT8sig)
     - TaqMan® Fast Advanced Master Mix
     - Amplification primers (Qfor8, Qrev8, L, M, G, B)
     - Probes (QRL8 [FAM], C [VIC], and U [NED])
     - Double stranded Internal Positive Control DNA (C/E)
   - STR Amplification Kits (i.e., Globalfiler, Globalfiler Express)
   - Y-STR Amplification Kits (i.e., Yfiler, Y23)
   - Mitochondrial DNA amplification and sequencing systems:
     - 10X PCR Buffer
     - BSA
     - Amplitaq Gold
     - Primers for mtDNA amplification and sequencing
     - Deoxyribonucleotide triphosphate mix (dNTPs)
     - Big Dye Sequencing Kits
     - HL60 DNA
   - EXOSap-IT
   - Amplification and Sequencing components (if not within a test kit or system):
     - Amplitaq Gold
     - Primers
     - Allelic Ladders
   - Rapid DNA Cartridge

B. The QC procedures used to ensure the reliability of critical reagents are contained in the appropriate DNA procedure or in STACS.

C. The results of the QC testing, as well as the reagent's acceptance or rejection for use, will be recorded. The reagent will be available for use once the necessary acceptance is recorded, generally in STACS.

4 SAFETY

A. 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.

B. Refer to the DNA Procedures Introduction (i.e., BIO-100) and/or the relevant DNA procedure for additional applicable safety information.

5 RECORDS

Records pertaining to the receipt, preparation, and/or QC of laboratory reagents will be kept in the STACS or an equivalent log or storage location. These records will be maintained by the DSU QA/QC Group and/or in the applicable DNA Unit. Records for commercial orders will be maintained by appropriate personnel.

6 REVISION HISTORY

Revision	Issue Date	Changes
00	09/15/22	Reformatted DNA 609-9 into new template and assigned new Doc ID. Minor edits throughout. Deleted obsolete reagent recipes.

7 APPENDIX A: REAGENT RECIPES AND CONTROL PREPARATION

7.1 Reagent Preparation Guidance:

A. If more or less reagent is needed than what is listed, the components should be adjusted proportionally to make the volume needed.
B. Graduated cylinders and/or pipettes closest in capacity to the volume of liquid being measured should be used.
C. If the pH meter is used, the performance will be verified prior to use.
D. Any reagent in which microbial growth is observed must be discarded.
E. Store all reagents in sterile containers unless otherwise noted.
F. When available, purchased ready to use reagents of equivalent or higher quality may be substituted for the reagents listed below.

7.1.1 1X Genetic Analyzer Buffer with EDTA

- Combine 100 mL of 10X genetic analyzer buffer with EDTA with 900 mL reagent grade water.
    - For a single 3130XL reservoir: Combine 3.5 mL 10X buffer with 31.5 mL water
- Store refrigerated for up to 1 month.

7.1.2 Acid Phosphatase Spot Test Solution, 100 mL

- Add 2.6 g Acid Phosphatase Spot Test powder to 100 mL reagent grade water and stir until dissolved.
- Store frozen for up to one month.

7.1.3 3% Bleach Solution, 100 mL (For TECAN only)

- Dilute 3 mL molecular grade bleach (or equivalent) to 100 mL with reagent grade water.
- Store at room temperature.
- Prepare daily.

7.1.4 10% Bleach Solution, 50 mL (for use on evidentiary items)

- Dilute 5 mL household bleach (or equivalent) to 50 mL with reagent grade water.
- Store at room temperature.
- Prepare daily.

7.1.5 10% Bleach Solution, 10 L (for cleaning purposes)

- Dilute 1 L household bleach (or equivalent) to 10 L with water.
- Store at room temperature.
- Prepare at least weekly.

7.1.6 Bovine Serum Albumin (BSA), 1.6 mg/mL, 80 mL

- Add less than 80 mL of reagent grade water to a container.

- Add 128 mg of bovine serum albumin.
- Bring final volume to 80 mL with reagent grade water.
- Store frozen.

7.1.7 Demineralization/Extraction Buffer, 1L

- Add 900 mL of 0.5M EDTA solution to a container and stir on medium.
- While stirring, add 10g of N-Lauroylsarcosine sodium salt.
- Allow mixture to go into solution.
- Adjust to pH 8.0 with hydrochloric acid (HCl) or Sodium Hydroxide (NaOH).
- Bring final volume to 1 L with 0.5M EDTA solution.
- Store at room temperature.

7.1.8 1M DTT (Dithiothreitol), 10 mL

- Dissolve 1.54 g of DTT in 10 mL of reagent grade water.
- Store frozen.

7.1.9 5M DTT (Dithiothreitol), 2 mL

- Dissolve 1.54 g of DTT in 2 mL of reagent grade water.
- Store frozen.

7.1.10 3% Hydrogen Peroxide Solution, 1 L

- Dilute 100 mL of 30% hydrogen peroxide solution to 1 L with reagent grade water.
- Store refrigerated.

7.1.11 mtDNA Amplification Primers

Calculation: The OD and Extinction Coefficient can be found on the certificate for each primer.

Optical Density (OD) (A260) / Extinction Coefficient (OD units/µmole) = µmole of primer

(100 µM = 0.0001 µmole/µL)

µmole of primer / 0.0001 µmole/µL = µL TE^{-4} for 100 µM solution

A. Prepare a 100 µM stock solution from lyophilized primer

- Add 1 to 2 mL of TE^{-4} to vendor tube with lyophilized primer (depending on tube size) and vortex.
- Let sit for ~5 min at room temperature and vortex again.
- Transfer liquid from vendor tube to a 50 mL conical tube.
- Add an additional 1 to 2 mL of TE^{-4} to vendor tube, vortex, and transfer to same 50 mL conical tube.

- Add remaining amount of TE^{-4} to bring solution to volume determined by calculation above. (Remember to subtract the initial 2-4 mL used)
- This will be the 100 µM stock primer solution (vortex before transferring to other tubes) used to prepare the 30 µM, 10 µM and 1 µM primer solutions as needed.

B. Prepare a 30 µM solution for primers A1, A2, B1, B2, C1, C2, D1, D2, 617 from the 100 µM stock solution and store frozen:

1. A1 primer:
   - Transfer 4.5mL of 100 µM stock solution to a new 50 mL conical tube.
   - Add 10.5 mL of TE^{-4}.

2. All other primers:
   - Transfer 3.15 mL of 100 µM stock solution to a new 50 mL conical tube.
   - Add 7.35 mL of TE^{-4}.

C. Prepare a 10 µM primer solution for all miniprimers from the 100 µM stock solution.
- Transfer 300 µL of 100 µM stock solution to a new 50 mL conical tube.
- Add 2700 µL of TE^{-4}.

7.1.12 mtDNA Quantitative PCR IPC - Double Stranded Internal Positive Control DNA (C/E)

- Reconstitute forward (C) and reverse (E) oligonucleotides in TE^{-4} buffer.
- Prepare 100 µM solutions of the forward and reverse oligonucleotides using information from vendor certificate of analysis.
- Combine these in equal volumes and tightly cap, mix, and quick spin the tube.
- This generates the **primary (1°) stock** of the double-stranded IPC DNA at 50 µM (3 x 10^{13} copies/µL). Store frozen.
- Prepare a dilution series using TE^{-4} as follows:
  - 2° stock: Transfer 10 µL of primary stock into 1,542 µL TE^{-4} (1.94 x 10^{11} copies/µL).
  - 3° stock: Transfer 10 µL of 2° stock into 1,542 µL TE^{-4} (1.25 x 10^{9} copies/µL).
  - 4° stock: Transfer 10 µL of 3° stock into 990 µL TE^{-4} (1.25 x 10^{7} copies/µL).
  - 5° stock: Transfer 10 µL of 4° stock into 990 µL TE^{-4} (1.25 x 10^{5} copies/µL).
  - 6° stock (working dilution): Transfer 10 µL of 5° stock into 990 µL TE^{-4} 1.25 x 10^{3} copies/µL).
- Store Frozen

7.1.13 mtDNA Quantitative PCR Primers (Forward [Qfor8, L, G] and Reverse [Qrev8, M, B])

- Reconstitute all primers in TE^{-4} buffer.
- Prepare 100 µM stock solutions of each primer using information from vendor certificate of analysis.

- Store frozen.

- Prepare working dilutions of each primer in TE^{-4} buffer as follows:
    - For Qfor8 and G (1.25 µM) transfer 12.5 µL of 100 µM stock solution to a new tube and add 987.5 µL of TE^{-4}.
    - For Qrev8 and M (22.5 µM) transfer 225 µL of 100 µM stock solution to a new tube and add 775 µL of TE^{-4}.
    - For L and B (7.5 µM) transfer 75 µL of 100 µM stock solution to a new tube and add 925 µL of TE^{-4}.
- Store frozen.

7.1.14 mtDNA Quantitative PCR Probes (QRL8 [FAM], C [VIC], U [NED])

- Prepare a 6.25 µM working dilution from each 100 µM probe stock.
    - Transfer 62.5 µL of 100 µM stock solution to a new tube and add 937.5 µL of TE^{-4}.
- Store frozen and protected from light as much as possible.

7.1.15 mtDNA Quantitative PCR Primer/Probe/IPC Mix (PPI Mix)

- Prepare working dilutions of all primers, probes, and IPC DNA.
- Add 80 µL of all primers, probes, and IPC DNA into each tube. Vortex, pulse spin.
- Store frozen.

7.1.16 mtDNA Quantitative PCR Standard - Double Stranded Synthetic Standard (dsT8sig)

- Reconstitute Tfor8sig and Trev8sig oligonucleotides in TE^{-4} buffer.
- Prepare 2 µM solutions of the forward and reverse oligonucleotides based on their respective molecular weights of 34,960.7 g/mol and 35,969.3 g/mol.
- Combine these in equal volumes and tightly cap, mix, and quick spin the tube.
- This generates the **primary (1°) stock** of the double-stranded **dsT8sig** standard at 1 µM (6.023×10^{11} copies/µL).
- Store frozen.

- Prepare the **secondary (2°) stock** of **dsT8sig** from the **1° stock** by adding 1194.6 µL TE^{-4} buffer to a 10 µL aliquot of the primary (1°) stock. Tightly cap, mix, and quick spin the tube. The **2° stock of dsT8sig** should be at a final concentration of 5×10^9 copies/µL.
- Prepare 10 µL aliquots of the **2° stock** and store frozen.

- Prepare the mtDNA Quantitiative PCR Standard Dilution Series by adding 990 µL TE^{-4} buffer to a 10 µL aliquot of the **2° stock.** This can be stored refrigerated and used for up to two months.
    - Tube A: Transfer 20 µL from diluted **2° stock** and add 80 µL TE^{-4} buffer.
    - Tube B: Transfer 10 µL from tube A and add 90 µL TE^{-4} buffer.
    - Tubes C-G: Transfer 10 µL from previous tube and add 90 µL TE^{-4} buffer.

7.1.17 <u>mtDNA Sequencing Primers (1 µM)</u>

- New lots of primers used in cycle sequencing will be diluted from the 100 µM primer stocks.
- Prepare a 1 µM solution from the 100 µM stock solution and store frozen:

A. A1 primer:
   - Transfer 240 µL of 100 µM stock solution to a new 50 mL conical tube.
   - Add 23,760 µL of TE^{-4}.

B. All other primers:
   - Transfer 150 µL of 100 µM stock solution to a new 50 mL conical tube.
   - Add 14,850 µL of TE^{-4}.

C. Miniprimers:
   - Transfer 50 µL of 100 µM stock solution to a new 50 mL conical tube.
   - Add 4950 µL of TE^{-4}.

7.1.18 <u>Phenolphthalin Solution, 1 L</u>

- Combine 4 g phenolphthalin, 40 g NaOH, and 200 mL reagent grade water.
- Add 800 mL of ethanol and mix.
- Store refrigerated in an amber bottle over zinc (generally enough to cover the bottom of the bottle).
- This solution may be used for up to 3 months.

7.1.19 <u>Quantifiler® TRIO DNA standard Calibrators</u>

- The calibrator samples will be prepared from the Quantifiler® Trio Standard Stock (~100 ng/µl).
   - Prepare a 1:10 dilution (~10 ng/µl) by adding 30 µl of the Quantifiler® Trio Standard Stock and 270 µl of TE-4.
   - Prepare a 1:50 dilution (~2 ng/µl) by adding 6 µl of the Quantifiler® Trio Standard Stock and 294 µl of TE-4.
- Calibrators will be stored frozen, until thawed, then may be stored refrigerated and used for up to two months.

7.1.20 <u>Saturated D-Glucose Solution</u>

- Dissolve 10 g of dextrose in 10 mL reagent grade water with mild heating.
- Store at room temperature for up to 1 month.

7.1.21 <u>2.5 M (10% w/v) Sodium Hydroxide (NaOH), 100 mL</u>

- Add 10 g of NaOH to 80 mL reagent grade water.
- Store at room temperature.

7.1.22 Stain Extraction Buffer (SEB), 1 L (10 mM Tris-HCl / 100 mM NaCl / 10 mM EDTA / 2% SDS, pH 8.0)

- Dissolve 5.84 g NaCl in approximately 500 ml reagent grade water.
- Add 10 mL 1M Tris-HCl.
- 20 ml 0.5M EDTA.
- Add 100 mL 20% SDS.
- Adjust to pH 8.0 with HCl.
- Adjust the final volume to 1 L with reagent grade water.
- Store at room temperature.

7.1.23 SEB with Dithiothrietol (SEB w/DTT), 5 mL (10 mM Tris-HCl / 100 mM NaCl / 10 mM EDTA / 2% SDS / 39mM DTT)

- Add 30 mg of DTT to 5 mL of SEB and stir until dissolved.
- Store at room temperature for up to 1 month.

7.2 Control Preparation Guidance:

7.2.1 Blood/Buccal Internal Standard (BIS)

An individual providing the BIS control must have a previously characterized and documented STR profile.

- A blood BIS control is an FTA bloodstain card prepared with liquid blood or blood collected via a finger stick.
- A buccal BIS control is the FTA card from a Whatman EasiCollect™ device which is used to collect and transfer a buccal sample to the card, the collector from a Bode Collector device used for a buccal collection, or an equivalent card collected using an approved collection device.

7.2.2 Blood Known Positive Swab

- Prepare a 1:20 dilution of whole blood.
- Add approximately 50uL of diluted blood to each clean swab.
- Allow the swabs to dry completely.
- Store refrigerated or at room temperature.

7.2.3 mtDNA Positive Control (HL60)

Calculation:

Concentration of HL60 (stock) x V1 = 10 ng/µL x 200 µL

V1 = amount of stock needed to make a 10 ng/µL solution

200 µL – V1 = amount of TE^{-4} needed to make a 10 ng/µL solution

A. Prepare a 10 ng/µL stock solution from the vendor stock of the HL60 DNA.
    - Add amount of stock and TE^{-4} determined by above calculation.
    - Store frozen.

B. Prepare a 20 pg/µL solution from the 10 ng/µL stock.
    - Add 19,960 µL of TE^{-4} to 40 µL of the 10 ng/µL solution.
    - Store frozen.

C. Prepare a 100 pg/µL solution from the 10 ng/µL stock.
    - Add 14,850 µL of TE^{-4} to 150 µL of the 10 ng/µL solution.
    - Store frozen.

7.2.4 Semen Known Positive Slide

- Prepare a 1:10 dilution of neat human semen with water.
- Pipette 4 µL of diluted semen to center of microscope slide.
- Allow slide to air dry (generally for 10 minutes).
- Store at room temperature.

7.2.5 Semen Known Positive Swab Preparation

- Add 1.5 mL of human semen to 3.0 mL of water.
- Add approximately 100 µL of diluted semen to each swab.
- Allow the swabs to dry completely.
- Store frozen.