1.1 PURPOSE:

1.1.1 To provide an overview of evidence handling, extraction, amplification, and post-amplification practices in the Mitochondrial DNA Unit.

1.2 RESPONSIBILITY:

1.2.1 Forensic Science Examiners from the CT DESPP Division of Scientific Services who have been trained in the discipline of Mitochondrial DNA Analysis according to the Mitochondrial DNA Unit Training Manual.

1.3 SAFETY:

1.3.1 Use appropriate measures for the proper handling of physical evidence according to the Laboratory Safety Manual.

1.4 Evidence Handling, Extraction, Amplification and Post-Amplification Practices

1.4.1 Extraction and amplification set-up areas are separated by space from post-amplification work areas. If an individual performs post-amplification work during a particular day, that individual will not conduct any extractions or amplification set-ups later in that day. Polymerase Chain Reaction (PCR) product and genomic DNA are separated by space. Amplified mtDNA will not be moved from the post-amplification work areas into the extraction/amplification set-up areas. Lab coats used in the post-amplification work areas will not be moved to the extraction/amplification set-up areas prior to laundering.

1.4.2 Dedicated lab coats, disposable gloves, clean room facemasks, protective eyewear (when appropriate), and other relevant personal protective equipment will be worn at all times during extraction and amplification set-up procedures. Dedicated lab coats and disposable gloves will be worn at all times during the post-amplification procedures. Gloves will be removed and appropriately discarded upon exiting post-amplification work areas when work is completed.

1.4.3 Pipettes, aerosol resistant pipette tips, scalpels, scissors, forceps, extraction, and amplification reagents are dedicated for use in mtDNA extraction/amplification set-up. Sterile scalpel blades, pipette tips, and other consumable supplies will be used (when appropriate) for each item. Forceps, scissors, and other non-consumables will be cleansed appropriately between items.

1.4. 4 All appropriate glassware and plastic containers will be autoclaved prior to use.

1.4. 5 Only one item of evidence from a case should be opened at a time. Items of questioned origin typically will be extracted and amplified prior to items of known origin.

1.4. 6 All extraction and amplification set-up procedures will be performed under a dedicated laminar flow hood or dead space hood that includes a UV light source. Bones and teeth will be cleaned and cut under dedicated hoods. Genomic DNA will be added to the PCR reactions in the laminar hood.

1.4. 7 Work surfaces and equipment in the extraction/pre-amplification areas will be cleaned with a 20% (in-house) bleach solution or 10% stabilized bleach. Refer to DNA WI-21 for instructions on in-house bleach preparation.

1.4. 8 Work spaces under the dedicated hoods in the extraction/amplification set-up areas will be cleaned with a 20% (in-house) bleach solution or 10% stabilized bleach and exposed to UV light for a minimum of 15 minutes before and after processing a sample. Bleach cleaning and UV illumination will be documented on appropriate log sheets located on each hood.

1.4. 9 All reagents and microfuge tubes must be opened with caution. At no time should open tubes of reagents be removed from the laminar flow hood.

1.4. 10 A reagent blank (RB) will be processed from extraction with each evidentiary sample. A negative control (NC) and positive control will be processed from amplification with each evidentiary sample.

1.4. 11 For all mixing and pipetting pertaining to PCR set-up, only the pipettes from the extraction/PCR set-up rooms will be used. At no time will these pipettes be removed from these rooms, except for calibration checks. Pipettes from other areas of the lab will not be brought into these rooms.

1.4. 12 Aerosol blocking pipette tips will be used at all times.

1.4. 13 A PCR master mix will be prepared and aliquoted to each tube prior to the addition of DNA.

1.4. 14 Amplified product will not be brought into the extraction/PCR set-up rooms.

1.4. 15 All PCR set-up will be performed using microfuge tube racks designated for amplification.

1.4. 16 Physical evidence in the mtDNA unit will be handled and documented according to standard CT DESPP Division of Scientific Services procedures and FBI QAS Standard 7. All case results are documented on the appropriate worksheets. Refer to DNA SOP 1 for instructions on the consumption of evidence.

1.4. 17 Extracts containing genomic DNA from local cases will be maintained in a locked freezer within the DNA unit. The boxes containing these extracts that have been filled will be sealed with tape and initialed.

Genomic DNA extract and amp product are considered to be work product- material generated as a function of analysis. They are not considered evidence; therefore, they are not sub-itemized in LIMS or given a barcode.

1.4.18 Hairs, bones and teeth typically will be extracted up to three times to obtain a DNA profile. More extractions may be done at the analyst's discretion. Two centimeters of hair is used per extraction, with analyst discretion to use less or more as appropriate.

Powder generated from the grinding and pulverization of bones and teeth is considered to be work product-material generated as a function of analysis. It is not considered evidence; therefore, it is not sub-itemized in LIMS or given a barcode.

1.5 Quality Control of Reagents

1.5.1 The following sections identify the critical reagents used in mtDNA analysis and describe quality control procedures to ensure suitability for casework. The quality of all reagents is verified for each method based on the performance of the appropriate control samples. Refer to GL-2 for labeling reagent bottles. Refer to mtDNA WI-16 for further instructions on reagent QC.

1.5.2 Extraction Critical Reagents

- 1.5.2.1 Stain Extraction Buffer (SEB)
- 1.5.2.2 Proteinase K
- 1.5.2.3 DTT (Dithiothreitol)
- 1.5.2.4 PCIA (Phenol/Chloroform/Isoamyl Alcohol 25:24:1 v/v)
- 1.5.2.5 AL, AW1, AW2, and AE buffers and ethanol in the QIAamp DNA Mini Kits

1.5.3 Amplification Critical Reagents

- 1.5.3.1 10X PCR Buffer
- 1.5.3.2 AmpliTaq Gold

1.5.3.3 dNTPs (individual or pre-mixed)
1.5.3.4 BSA
1.5.3.5 Positive Control HL60
1.5.3.6 Amplification primers
1.5.3.7 ExoSAP-IT™

1.5.4 Sequencing Critical Reagents/Equipment
1.5.4.1 Big Dye v1.1
1.5.4.2 Sequencing Primers
1.5.4.3 Capillary Arrays for the ABI Prism™ 3130 Genetic Analyzer
1.5.4.4 Formamide
1.5.4.5 POP-6

1.5.5 The critical reagents are deemed suitable for casework after extracting, amplifying, and sequencing known control samples (i.e. positive and negative controls) and achieving the expected results. Sequence data is confirmed by results from the light (L) and heavy (H) strands. A known blood sample (EP1) and reagent blank are used for QC testing extraction reagents. A negative control and HL60 are used for QC testing amplification and sequencing reagents. The controls are tested through sequencing of HV1 or HV2. See protocol section 14 Appendix A for the expected results of EP1 and HL60. Extraction reagent blanks and amplification negative controls are expected to show no interpretable sequence. In case of results other than those expected or poor quality data, the reagents will not be used until the problem is identified and corrected. If the problem cannot be resolved, the reagents will not be used for casework and fresh reagents will be made and tested. All quality documentation for reagents is kept in a dedicated binder. The status of each reagent is noted in this documentation, i.e. the reagent did or did not perform as expected. Reagents are not used for casework analysis past their control date. Reagents may be used for research/validation purposes past their control date where appropriate. Refer to mtDNA WI-16 for more information on reagent QC.

1.6 Equipment Calibration and Maintenance

1.6.1 All equipment used for casework analysis is maintained in proper working condition. Any equipment that is in need of repair or is out of calibration will not be used for casework until repairs/calibration are completed. The Agilent 2100 Bioanalyzers and the 3130 Genetic Analyzer will be performance checked following yearly maintenance/calibration and repairs. The 9700 thermal cyclers will be performance checked following repairs. See mtDNA WI-17 for information regarding performance checks. Also, see mtDNA SOP-12 for a list of mtDNA equipment and procedures for calibration and maintenance.

1.7 NIST Standard Reference Material

1.7.1 The extracted human DNA components of the NIST Standard Reference Material 2392-I (HL60) are amplified and sequenced at HV1 and HV2. Sequence results are checked for accuracy. Documentation of the results is maintained in the designated QC binder.

1.8 Interpretation of Control Samples

1.8.1 Reagent Blank (RB)

1.8.1.1 If the amount of amplified mtDNA in the RB exceeds 10% of the amount of amplified mtDNA in the evidentiary sample (assessed by CE quantification), the RB may be sequenced to determine a possible source of the sequence. The sample will be re-amplified or re-extracted as appropriate.

1.8.1.2 If the amount of amplified mtDNA in the RB does not exceed 10% of the amount of amplified mtDNA in the evidentiary sample (assessed by CE quantification), then the RB and sample sequences can be used for comparison purposes unless the RB and evidentiary sample are concordant. If the RB sequence is in concordance with the evidentiary sample, the results of the sample amplification reaction are not used for comparison purposes. The sample will be re-amplified or re-extracted as appropriate.

1.8.2 Negative Control (NC)

1.8.2.1 If the amount of amplified mtDNA in the NC exceeds 10% of the amount of amplified mtDNA in the evidentiary sample (assessed by CE quantification), the NC may be sequenced to determine a possible source of the sequence. The sample will be re-amplified or re-extracted as appropriate.

1.8.2.2 If the amount of amplified mtDNA in the NC does not exceed 10% of the amount of amplified mtDNA in the evidentiary sample (assessed by CE quantification), then the NC and sample sequences can be used for comparison purposes unless the NC and evidentiary sample are concordant. If the NC sequence is in concordance with the evidentiary sample, the results of the sample amplification reaction are not used for comparison purposes. The sample will be re-amplified or re-extracted as appropriate.

1.8.3 Positive Control HL60

1.8.3.1 If the positive control fails to amplify or sequence correctly, re-amplify (per region) and/or

re-sequence (per primer) the sample set to include the NC, RB, sample, and HL60 as necessary. Refer to mtDNA SOP-14 for the correct sequence of HL60.

1.9 Preparation of Discovery Materials

1.9.1 Discovery materials will be made available to authorized individuals according to CT DESPP Division of Scientific Services guidelines. The Director of the CT DESPP Division of Scientific Services or designee must approve the release of any case materials. Any sample information not pertaining to the case in question will be obliterated. The requested information will be duplicated in an appropriate manner and a copy of all released material will be retained at the CT DESPP Division of Scientific Services. Release of case material will be noted in the Main Case File. A fee may be charged to cover administrative costs.

1.10 mtDNA Analysis Work Flow

1.10.1 Upon receipt of any item for mtDNA analysis, the first step is to document all items and the packaging they are contained in. This documentation includes written, photographic, and/or photocopied means. The DNA is then extracted and amplified using the Polymerase Chain Reaction (PCR). The quantity of mtDNA is estimated using an Agilent 2100 Bioanalyzer. The amplified DNA product is sequenced using a validated sequencing chemistry. Other validated methods may be used on a case-by-case basis.

1.11 Report Writing

1.11.1 DNA results are reported according to standard CT DESPP Division of Scientific Services guidelines. Final reports will include mtDNA typing results, a qualitative interpretative statement (see report templates, mtDNA SOP-10 and DNA SOP-06 where appropriate), and statistical analysis for each item with a "cannot be excluded" result. The signature on the left is the analyst responsible for the case. The signature on the right is the technical reviewer of the case. All items tested in the mtDNA unit are included in the report.

1.12 Contamination

1.12.1 Contamination is defined as the introduction of a secondary source of DNA (genomic or amplified) into a sample at the CT DESPP Division of Scientific Services. This is to be distinguished from sample mixtures or contamination at the time of collection, which may require different action. If contamination is detected, review the results and attempt to determine the source of the contamination. Perform appropriate corrective measures as warranted by the nature/source of the contamination.

Potential contamination is assessed by evaluating the evidentiary samples, RB, NC, and positive control (see section 1.8). After following the steps outlined in section 1.8, an incident of contamination will be defined as the amount of amplified mtDNA in the NC and/or RB that exceeds 10% the amount of amplified mtDNA in the evidentiary sample OR the amount of amplified mtDNA in the NC and/or RB which is less than 10% the amount of the evidentiary sample but is the same mtDNA sequence as that of the evidentiary sample. Each incident of contamination will be documented and maintained by the Quality Section with access by the TL. The TL will be notified of all incidents of contamination. If a QAR is required (as outlined in Quality Manual GL-9), it will be approved by the TL.

1.12.2 Discard any reagents that are contaminated.

1.12.3 Thoroughly clean the PCR set-up/DNA extraction areas as needed.

1.12.4 All mtDNA unit personnel must supply a known sample for elimination purposes prior to working in the lab.

1.13 Monitoring, cleaning, and decontaminating facilities and equipment

1.13.1 DNA testing facilities and equipment are monitored, cleaned, and decontaminated (when applicable) per FBI QAS (Standard 6) and laboratory SOPs.

1.13.2 Document that the laboratory cleaning procedures were followed for all pre-amplification processes using worksheet QRM-12.

1.14 Case documentation

1.14.1 All case results are documented on the appropriate worksheets (mtDNA workbook, QRM-1 through 7). All photographs are included on the relevant worksheets. For mtDNA analysis, the Review Checklist (mtDNA workbook, QRM-8), Database Search results (when applicable), Difference Review documentation (when applicable), and Sequencher Projects (when applicable) are included in the case folder. For CODIS entries (when applicable), the DNA-QR-13A CODIS Profile Entry-Missing Persons form will be included in the case folder. All electronic files regarding mtDNA analysis are archived on optical disks.

1.15 Case review

1.15.1 All mtDNA case files are technically reviewed by at least one mtDNA analyst in addition to the primary analyst. All mtDNA case files must be administratively reviewed by a member of the DNA unit other than the primary analyst and the technical reviewer.
The primary analyst will perform a technical and administrative review of the case file and initial/date the review checklist worksheet (QRM-8) to show the review has been completed. The technical reviewer will perform a technical and administrative review of the case file and check off the corresponding boxes on the worksheet (QRM-8). The technical reviewer will initial/date the review checklist as well as the outside of the case file. The administrative reviewer will perform an administrative review of the case file and check off the corresponding boxes on the worksheet. The administrative reviewer will initial/date the worksheet as well as the outside of the case file. After all necessary corrections are made by the primary analyst (where appropriate), the technical reviewer and administrative reviewer will check off the corresponding box on the worksheet. The technical reviewer will co-sign the final report once all the reviews are completed and corrections are made. Refer to DNA SOP-14 for further instructions when a database upload is performed.

1.15.2 In the event of discrepant conclusions, the two analysts will discuss/review the results. Should a discrepancy persist, the data will be reviewed and conclusions approved by the technical leader.

1.16 Corrective Action

1.16.1 Where warranted, corrective action will be taken as outlined in the Quality Manual. Any corrective action in the mtDNA unit shall be approved by the TL prior to implementation. The TL has the authority to initiate, suspend, and resume mtDNA analytical operations for the mtDNA unit or an individual.

1.17 Laboratory Safety protocols

1.17.1 Laboratory safety protocols are outlined in the CT DESPP Division of Scientific Services Safety Manual.

1.18 Quality Manual

1.18.1 General CT DESPP Division of Scientific Services protocols are outlined in the Quality Manual & Laboratory Directives (current version).

1.19 DNA Analyst Training and Continuing Education

1.19.1 All mtDNA analysts are trained prior to assuming casework duties as outlined in the Quality Manual, the mtDNA Training Manual (DNA SOP-07), and FBI QAS (Standard 5). mtDNA analysts will successfully complete a competency test prior to independent casework analysis. The TL is responsible for the oversight and approval of training in the mtDNA unit. The TL will assess and document any adjustments to the established training program with the aid of the Training Coordinator. Transcripts and educational qualifications of all analysts are maintained in a binder by the Training Coordinator which are approved and documented by the TL.

1.19.2 All mtDNA analysts will undergo two external proficiency tests semi-annually as outlined in the Quality Manual and FBI QAS (Standard 13). The TL is responsible for the oversight and approval of proficiency testing in the mtDNA unit. Proficiency tests results will be documented on the DNA Proficiency Testing Review Sheet (GL16:3). Documentation will be kept in the Proficiency Test Record Binder by the Quality Manager.

1.19.3 All mtDNA analysts will accumulate at least one continuing education unit (8 hours) and read at least one relevant scientific paper each year as outlined in FBI QAS (Standard 5). This record will be documented in the analyst's continuing education file and approved by the TL (see DNA section QR documents 30 and 31).

1.20 Method Validation

1.20.1 DNA analysis methods will be validated prior to use in casework according to FBI QAS (Standard 8) and the Quality Manual. Summaries must be written for method validations and approved by the TL. All SOPs and work instructions are approved by the TL. Other procedures approved by the TL may be used on a case-by-case basis.

1.21 Job Descriptions and Flow Chart

1.21.1 The mtDNA unit is overseen by the DNA unit Technical Leader. CODIS, as it pertains to mtDNA, is overseen by the CODIS Administrator. The Training Coordinator oversees training for the entire DNA section with approval by the TL. Refer to the nuclear DNA SOP-17 for job descriptions of the Technical Leader, CODIS Administrator, and Training Coordinator in addition to the flow chart relative to the DNA unit.

mtDNA analyst: An analyst in the mtDNA unit is an employee that has successfully completed the laboratory's training requirements for casework sample analysis (see the mtDNA Training Manual), passed a competency test, and has entered into a proficiency testing program as outlined in FBI QAS (Standard 13).

Daily duties include performing examinations on forensic samples submitted for mtDNA analysis, interpreting data, reaching conclusions, preparing reports, and providing testimonies (when needed).

Additional duties may include training new employees in the mtDNA Unit, participating in the research and validation of new technologies, technically and administratively reviewing other analyst's work, conducting QC testing on reagents and equipment, importing profiles into the National Missing Persons Database, and giving presentations to the forensic community on the topic of mitochondrial DNA analysis.

1.22 **Work Instructions**

1.22.1 Work instructions are documents containing detailed instructions that specify exactly what steps to follow to carry out an activity. Work instructions are more detailed than SOPs and are only created if more detailed instructions are necessary. Work instructions will be referenced in specific SOPs and will be treated as controlled documents.

1.23 **Audits, Outsourcing, and Facilities/Security**

1.23.1 Refer to DNA SOP-1 for information on audits, outsourcing, and facilities/security.

1.24 **Additional Information Required for Requests for Missing Persons Testing**

To better facilitate the processing of Missing Persons cases, the following form will be utilized by agencies submitting evidence in the categories of unidentified human remains, missing person, and/or biological relative. See form on internet site and attached below: http://www.ct.gov/despp/lib/despp/dss/forms/missing_persons_form_sop-dna.pdf

mtDNA SOP-01:1
Revision 01

STATE OF CONNECTICUT

DEPARTMENT OF
EMERGENCY SERVICES and PUBLIC PROTECTION
DIVISION OF SCIENTIFIC SERVICES

DESPP

Guy M. Vallaro, Ph.D.
Director

Laboratory #: _____

Additional Information Required for Requests for Missing Persons Testing

To better facilitate the processing of Missing Persons cases, please provide the following information in addition to the case summary described on the DESPP Request for Examination of Physical Evidence. Please submit this form at the time of evidence submission. A new form should be completed each time additional evidence is submitted.

Submitting Agency: _____ Agency Case #: _____

1) If available, please list the following:
   a. NamUs #: _____
   b. VICAP #: _____
   c. NCIC #: _____

2) Are other family members willing to submit a sample for DNA testing? If so, who are they and what relationship do they have to the missing individual?
   a. Name: _____ Relationship: _____
   b. Name: _____ Relationship: _____
   c. Name: _____ Relationship: _____

3) Please list any metadata specific to the corresponding category:
   a. Unidentified person (age range/age at time of death, height, sex, ethnic group scars/marks/tattoos, date of recovery, location of recovery)

   b. Missing person (date of birth, height, sex, ethnic group, scars/marks/tattoos, location of last contact to include city and state)

   c. Biological relative (sex, ethnic group)

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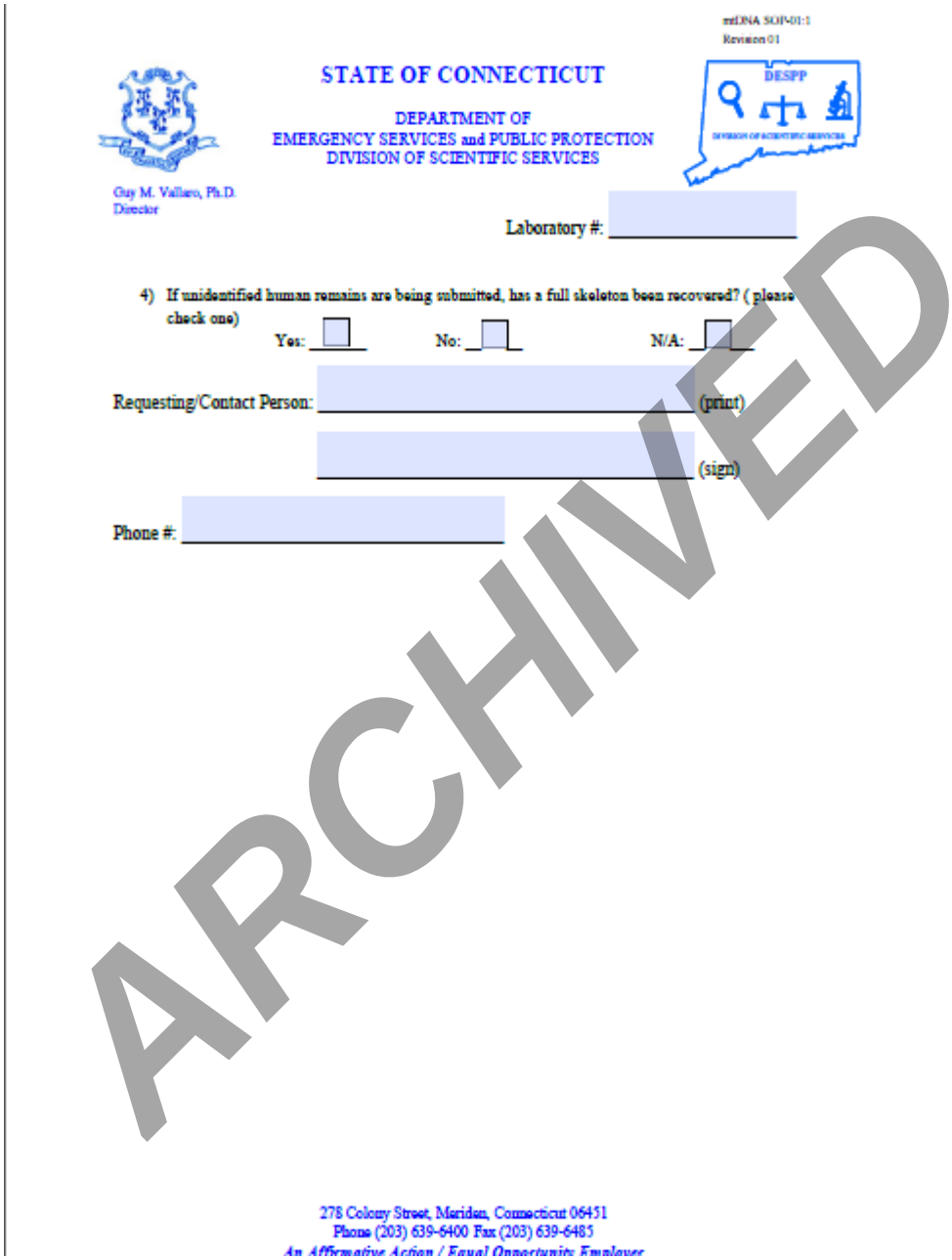

mtDNA SOP-01:1
Revision 01

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4) If unidentified human remains are being submitted, has a full skeleton been recovered? (please check one)

Yes: ____ No: ____ N/A: ____

Requesting/Contact Person: _____ (print)

_____ (sign)

Phone #: _____

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