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# Standard for Training in Mitochondrial DNA (mtDNA) Analysis for Taxonomic Identification

*410 North 21st Street, Colorado Springs, CO 80904, www.asbstandardsboard.org*

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# Foreword

This document addresses the need in wildlife forensics to provide minimum criteria for a training program for wildlife forensic DNA analysts who will be conducting taxonomic identification of animal material. This document outlines training in taxonomy and phylogeny and their impact on determination of an evidence item's taxonomic origin. This standard mandates training in an array of practical laboratory skills needed to address both the diversity of species and source materials encountered in wildlife forensic casework. This document does not address the specific educational and skill requirements for a wildlife forensic analyst, as it is expected that the employing agency will have established criteria for specialized employment categories.

This document was revised, prepared, and finalized as a standard by the Wildlife Forensics Consensus Body of the AAFS Standards Board. The draft of this standard was developed by the Wildlife Forensics Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science.

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>
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All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

**Keywords:** *Training, Mitochondrial DNA, Wildlife, Taxonomic Identification.*

# Table of Contents

# Standard for Training in Mitochondrial DNA (mtDNA) Analysis for Taxonomic Identification

## 1 Scope

This standard provides requirements to ensure proper training in animal taxonomic identification based on mitochondrial DNA (mtDNA) sequencing, data analysis, and reporting within the trainee's forensic DNA laboratory.

## 2 Normative References

The following documents are indispensable for the application of the standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs*, First Edition, 2019[a]

ANSI/ASB Standard 023, *Standard for Training in Forensic DNA Isolation and Purification Methods*, First Edition, 2020[a]

## 3 Terms and Definitions

For the purpose of this document the following definitions apply.

**3.1**
**biogeography**
The study of the geographical distribution of living things.

**3.2**
**Basic Local Alignment Search Tool**
**BLAST**
Refers to a) the BLAST algorithm, and b) a suite of database search programs that implement variations of this algorithm to generate alignments between a nucleotide or protein sequence in a query, and nucleotide or protein sequences within a database.

**3.3**
**Barcode of Life Database**
**BOLD**
Nucleotide sequence database devoted to taxonomic studies and online platform for DNA sequence analysis of cytochrome oxidase I data.

**3.4**
**coalescence**
A model in population genetics to describe a process of how gene variants sampled from a population may have originated from a common ancestor.

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[a] Available from https://www.asbstandardsboard.org/published-documents/

**3.5**

**deamination**

The removal or loss of an amine group from a base, which can result in incorrect pairing during DNA amplification.

**3.6**

**DNA sequence**

The relative order of base pairs, whether in a fragment of DNA, a gene, a chromosome, or an entire genome.

**3.7**

**GenBank**

A public repository of DNA sequences maintained by the National Center for Biotechnology Information, part of the U.S. National Institutes of Health.

**3.8**

**heteroplasmy**

The presence of more than one mitochondrial DNA sequence or type within a single individual.

**3.9**

**homopolymeric DNA (homopolymer)**

A region of DNA made up of a single repeated base.

**3.10**

**hotspot**

A base or region of DNA that mutates more frequently than areas around it.

**3.11**

**hybridization**

Interbreeding between two taxa that are considered to have diverged in their genetic makeup and taxonomic relationship (e.g., between members of different families, genera, or species). Hybridization commonly occurs between subspecies or closely related species.

**3.12**

**incomplete lineage sorting**

A concept in evolutionary theory in which genetic isolation leads to the divergence of descendant species from their common ancestors. Incomplete lineage sorting is reflected in the retention of shared ancestral genetic or morphological characteristics between closely related species.

**3.13**

**in-house database**

A database of reference material or sequences assembled internally by a forensic laboratory.

**3.14**

**introgression**

The incorporation of genes of one species into the gene pool of another species as a result of successful hybridization and back crossing.

**3.15**
**maternal inheritance**
Any genetic information passed from the mother to the offspring.

**3.16**
**Mendelian inheritance**
A reference to the basic principles that govern the transmission of genetic traits from parents to offspring discovered by Gregor Mendel in the late 19th century.

**3.17**
**mitochondria, mitochondrial DNA (mtDNA)**
Organelles found in eukaryotes (including humans) that provide the energy for the cell; contains DNA inherited from an individual's biological mother.

**3.18**
**mutation rate**
The relative frequency at which mutations have been observed at a specific genetic locus; generally estimated as the number of mutations observed in parent-offspring pairs divided by the total number of pairs examined.

**3.19**
**Nuclear mitochondrial DNA segments**
**NUMTs**
Regions of mitochondrial DNA inserted into the nuclear genome over evolutionary time.

**3.20**
**phylogenetic systematics**
Branch of biology that focuses on the reconstruction of evolutionary history and patterns of relationships among organisms.

**3.21**
**phylogram**
A tree-like branching diagram that depicts the hypothesized evolutionary history among a group of organisms. The tips of the branches represent groups of descendant taxa, whereas the intersections between branches represent common ancestors.

**3.22**
**population genetics**
The study of genes and allele frequencies in populations.

**3.23**
**reference sequence(s)**
A DNA sequence used as a reference to describe variants that are present in a sequence being analyzed.

**3.24**
**sequencing, DNA**
A laboratory technique used to determine the sequence of bases (A, C, G, and T) in a DNA molecule.

**3.25**
**species**
Level of taxonomic classification between subspecies and genus.

**3.26**
**taxonomic identification**
Analyses to establish the classification of an organism to family, genus, species, etc. These analyses are based on class characters which are diagnostic for the taxonomic level in question.

**3.27**
**taxonomy**
A branch of science concerned with classification of organisms, including systematics and nomenclature.

**3.28**
**transition**
A mutation that results in a change from one purine to the other purine (e.g., A to G) or one pyrimidine to the other pyrimidine (e.g., C to T).

**3.29**
**transversion**
A mutation that results in a change from a purine to pyrimidine or vice versa (e.g., A to T).

## 4 Requirements

### 4.1 Knowledge-based Training

**4.1.1** The laboratory's training program shall provide the trainee with an understanding of the fundamental principles of DNA sequencing for taxonomic identification of animal materials, including the laboratory's own DNA interpretation guidelines.

**4.1.2** At a minimum, the knowledge-based portion of the training program shall require review of the following:

a) the laboratory's protocols for taxonomic identification from mtDNA sequences;

b) the laboratory's applicable validation studies;

c) literature used to support validation;

d) applicable literature as assigned by the trainer (e.g., Annex A).

**4.1.3** At a minimum, the knowledge-based portion of the training program shall cover the topics in 4.1.3.1 through 4.1.3.7.

**4.1.3.1** General biology of animal mtDNA, including topics identified in ANSI/ASB Standard 023, *Standard for Training in Forensic DNA Isolation and Purification Methods*, 4.1.2.1 a), b); to include the following topics.

a) Location-mtDNA vs nucDNA:

  - influence on DNA quantity and quality.

b) Structure; to include the following topics:

  - circular vs linear;
  - codons;
  - homopolymeric regions;
  - repeat regions;
  - heteroplasmy;
  - nuclear mitochondrial DNA segments (NUMTs).

c) Gene composition and order:

  - differences among higher order taxa.

d) Copy number.

e) Inheritance; to include the following topics:

  - maternal inheritance;
  - Mendelian inheritance.

f) Mutation; to include the following topics:

  - rates;
  - hotspots;
  - transitions/transversions;
  - insertions/deletion;
  - deamination;
  - mutation rate heterogeneity among lineages.

**4.1.3.2** Evolutionary theory; to include the following topics:

a) species concepts;

b) principles of taxonomy;

c) principles of phylogenetic systematics;

d) principles of biogeography;

e) principles of population genetics;

f) hybridization, incomplete lineage sorting, and introgression;

g) coalescence;

h) principles of interspecific and intraspecific variation.

**4.1.3.3** Sequence alignment; to include the following topics:

a) sequence alignment software;

b) sequence editing and removal of primer sequence;

c) reference sequences;

d) interpretation of length heteroplasmic sequences.

**4.1.3.4** Evaluation of controls shall conform with ANSI/ASB Standard 023 and Section 4.1.2.1 g), h); to include the following topics:

a) reagent blanks;

b) negative controls;

c) positive controls.

**4.1.3.5** Comparison of evidentiary data to known reference data; to include the following topics:

a) criteria for identification at the species level;

b) criteria for identification at higher taxonomic levels;

c) distance-based models;

d) Bayesian models;

e) phylograms.

**4.1.3.6** Databases; to include the following topics:

a) public databases such as BLAST/GenBank, Barcode of Life (BOLD) and others as appropriate;

b) in-house databases.

**4.1.3.7** Reporting of conclusions.

## 4.2 Practical Training

The laboratory's training program shall provide the trainee with sufficient practical instruction for the trainee to obtain the skills for performing mtDNA sequence-based taxonomic identification.

**4.2.1** At a minimum, the practical portion of the training program shall include the observation of the processes at least once, and until clearly understood. These include:

a) mtDNA sequence-based taxonomic identification of animal material to be examined by the trainee;

b) documentation of the process.

**4.2.2** At a minimum, the practical portion of the training program shall include exercises representative of the range, type, and complexity of routine casework or database samples processed by the laboratory and supplemented with case file review. These exercises include:

a) mtDNA sequence-based taxonomic identification of animal material performed by the trainee;

b) documentation of the process by the trainee;

c) review of work performed by another trained analyst;

d) a range of exercises that demonstrates the trainee's ability to follow the laboratory's mtDNA sequence-based protocols for animal taxonomic identification so as to produce reliable and accurate results.

## 4.3 Competency

The laboratory's training program shall culminate in knowledge-based and practical competency in the application of mtDNA-based taxonomic identification of animal material. The format of the test(s) shall meet section 4.3 of ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs.*

## 4.4 Knowledge-Based Competency

The trainee shall successfully complete a knowledge-based test covering the critical information obtained during the training for mtDNA sequence-based taxonomic identification of animal material. The test(s) shall cover, at a minimum:

a) the theoretical and scientific bases of mtDNA sequence-based taxonomic identification of animal material;

b) the function of the controls used in mtDNA sequence-based taxonomic identification of animal material with regard to interpretation and comparison of the sample results;

c) the quality control steps pertaining to mtDNA sequence-based taxonomic identification of animal material;

d) the laboratory's procedures pertaining to mtDNA sequence-based taxonomic identification of animal material.

### 4.5 Practical Competency

The trainee shall successfully complete a practical test covering the mtDNA sequence-based taxonomic identification of animal material protocol(s) for which he or she will be independently authorized. The trainee shall be able to satisfactorily perform the following, as applicable:

a) properly and accurately execute the procedures for mtDNA sequence-based taxonomic identification of animal material procedures;

b) apply the laboratory's mtDNA sequence-based taxonomic identification of animal material procedures to a variety of evidentiary casework- or database-type samples that are representative of those likely to be encountered in the laboratory;

c) operate relevant software used in the laboratory for mtDNA sequence-based taxonomic identification of animal material;

d) document work performed in accordance with laboratory procedures.

## 5 Conformance

In order to demonstrate conformance with this standard, the laboratory shall meet Section 5 of ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs.*

# Annex A

## (informative)

## Supplemental Reading Material

The following information provides a list of the literature resources that may assist the technical leader in defining the breadth and scope of the materials to be reviewed by the trainee. This is not meant to be an all-inclusive list; other publications on this subject exist. The laboratory shall develop a list tailored to its specific needs. Updated references shall be added to the laboratory's list as new methods or technologies are incorporated into the laboratory's protocols.

1] Ewart, K.M., G.J. Frankham, R. McEwing, L.M.I. Webster, S.A. Ciavaglia, A.M.T. Linacre, D.T. The, K. Ovouthan, R.N. Johnson. "An internationally standardized species identification test for use on suspected seized rhinoceros horn in the illegal wildlife trade." *Forensic Science International Genetics,* 2018, Jan;32:33–39.

2] Folmer, O., M. Black, W. Hoeh, Lutz, and R. Vrijenhoek. "DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates." *Molecular Marine Biology and Biotechnology.* 1994, 3(5): 294–299.

3] Foran, D. "The relative degradation of nuclear and mitochondrial DNA: An experimental approach." *Journal of Forensic Sciences.* 2006, 51, 766–770.

4] Foran, D.R., A.B. Fischer, and M.E. Stoloff. "A comparison of mitochondrial DNA amplification strategies for species identification." *Journal of Forensic Investigation*. 2015, 3, 1–7.

5] Kanthaswamy, S. "Review: domestic animal forensic genetics—biological evidence, genetic markers, analytical approaches and challenges." *Animal Genetics.* 2015, 46, 473–484.

6] Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Pääbo, F.X. Villablanca, and A.C. Wilson. "Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers." *Proceedings of the National Academy of Sciences USA*. 1989, 86(16): 6196–6200.

7] Lopez, J.V., N. Yuhki, R. Masuda, W. Modi, S.J. O'Brien. "Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat." *Journal of Molecular Evolution.* 1994, Aug;39(2):174–190.

8] Palumbi, S, M. Kewalo, S. Romano, W.O. McMillan, L. Stice, G. Grabowski. *Simple Fool's Guide to PCR*, 2000 Version 2.0. University of Hawaii.

9] Roca, A.L., N. Georgiadis, S.J. O;Brien. "Cyto-nuclear genomic dissociation and the African elephant species question." *Quaternary International.* 2007, Jul; 169-170: 4–16.

10] Ross, H.A., S. Murugan, W. Lok, S. Li. "Testing the Reliability of Genetic Methods of Species Identification via Simulation." *Systematic Biology*, 2008, Volume 57, Issue 2, 216–230.

11] Sorenson, M.D., J.C. Ast, Dimcheff, T. Yuri, D.P. Mindell. “Primers for a PCR-Based Approach to Mitochondrial Genome Sequencing in Birds and Other Vertebrates.” *Molecular Phylogenetics and Evolution* 1999, 12(2): 105–114.

12] Tobe S.S., A.C. Kitchener, A,M.T. Linacre. “Reconstructing Mammalian Phylogenies: A Detailed Comparison of the Cytochrome b and Cytochrome Oxidase Subunit I Mitochondrial Genes. PLoS One.” 2010;5(11):e14156. doi: 10.1371/journal.pone.0014156.

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