*This document has been accepted by the Academy Standards Board (ASB) for development as an American National Standard (ANS). For information about ASB and their process please refer to asb.aafs.org. This document is being made available at this stage of the process so that the forensic science community and interested stakeholders can be more fully aware of the efforts and work products of the Organization of Scientific Area Committees for Forensic Science (OSAC). The documents were prepared with input from OSAC Legal Resource Committee, Quality Infrastructure Committee, and Human Factors Committees, as well as the relevant Scientific Area Committee. The content of the documents listed below is subject to change during the standards development process within ASB, and may not represent the contents of the final published standard. All stakeholder groups or individuals, are strongly encouraged to submit technical comments on this draft document during the ASB's open comment period. Technical comments will not be accepted if submitted to the OSAC Scientific Area Committee or Subcommittees.*

# Wildlife Forensics Validation Standards—Sequencing

DRAFT

**DRAFT DOCUMENT**

ASB Standard 00XX
2017

# Wildlife Forensics Validation Standards—Sequencing

**Keywords:** wildlife forensics, validation, DNA sequencing, mitochondria, chromosome

**Abstract**
This document provides minimum standards and recommendations for validating new primers for mitochondrial haplotyping and/or taxonomic identification via sequencing in wildlife forensic DNA laboratories where the sequencing (Sanger) method has already been validated.

DRAFT

## Foreword

This document addresses the particular need in wildlife forensics to validate new primer sets for sequencing applications. This is for laboratories that have previously validated a DNA sequencing method and are developing new primers to be used with that method. Since expansions of methodology are based on previously validated foundational principles, methods, and reagents, the validation process for an expansion often requires a truncated developmental validation process and an internal validation. Because these two validations are completed in the same laboratory, the overlap between developmental and internal validation is extensive. Therefore, these standards cover validation, generally, and do not delineate between developmental and internal validation.

This validation document is needed to accommodate the diversity of species and substrates encountered in wildlife forensic laboratories. Differences in evolutionary histories for different taxonomic groups often necessitate the use of multiple primer sets for multiple loci. Laboratories need to be able to validate new primer sets in a straightforward and clearly defined way since this is a regular occurrence. Existing developmental and internal validation documents are all-encompassing and further guidance and details are required for laboratories who routinely need to validate new primer sets for sequencing.

This standard was developed in the OSAC Wildlife Subcommittee Validation Task Group, reviewed by the OSAC Wildlife Subcommittee and presented to the Biology Subject Area Committee for movement through the American Academy of Forensic Sciences' Academy Standards Board.

DRAFT

## Acknowledgements

Editor: **Christina Lindquist, M.S.,** UC Davis Veterinary Genetics Laboratory Forensic Unit

Deputy Editor(s):

Draft Working Group Members:

**Tasha Bauman*, M.S.,** Wyoming Game and Fish Wildlife Forensic and Fish Health Laboratory
**Mary Burnham-Curtis*, Ph.D.,** U.S. Fish & Wildlife Service, National Fish & Wildlife Forensics Laboratory
**Jason Byrd, Ph.D.,** University of Florida Maples Center for Forensic Medicine
**Brandt Cassidy*, Ph.D.,** DNA Solutions
**Seth Faith, Ph.D.,** NC State University
**David Foran, Ph.D.,** Michigan State University
**Kimberly Frazier*, M.S.,** Wyoming Game and Fish Wildlife Forensic Lab
**Jenny Giles*, Ph.D.,** Stanford University
**Brian Hamlin*, B.S.,** U.S. Fish & Wildlife Service, National Fish & Wildlife Forensics Laboratory
**Trey Knott*, M.S.,** NOAA/National Marine Fisheries Service/Marine Forensics Laboratory
**Christina Lindquist*, M.S.,** UC Davis Veterinary Genetics Laboratory Forensic Unit
**M. Katherine Moore, M.S.,** NOAA/National Marine Fisheries Service/Marine Forensics Laboratory
**Chris O'Brien, Ph.D.,** University of New Haven

*These members served on the original OSAC Wildlife Forensics Validation Task Group.

Consensus Group Members:

DRAFT

## Table of Contents


1 Scope .................................................................... 8

2 Normative References .................................................................... 8

3 Terms and Definitions .................................................................... 8

4 Standards .................................................................... 11

Annex A (informative) Bibliography .................................................................... 13


DRAFT

# Wildlife Forensics Validation Standards—Sequencing

## 1 Scope

This document provides minimum standards and recommendations for validating new primers for mitochondrial haplotyping and/or taxonomic identification via sequencing in wildlife forensic DNA laboratories where the sequencing (Sanger) method has already been validated.

## 2 Normative References

The following referenced documents are indispensable for the application of this document.

European Network of Forensic Science Institutes (ENFSI). Recommended Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process.

http://www.enfsi.eu/sites/default/files/documents/minimum_validation_guidelines_in_dna_profiling_-_v2010_0.pdf

SWGDAM. SWGDAM Validation Guidelines for Forensic DNA Analysis Methods.
http://media.wix.com/ugd/4344b0_cbc27d16dcb64fd88cb36ab2a2a25e4c.pdf

## 3 Terms and Definitions

**3.1** An amplicon is any PCR amplification product.

**3.2** Amplification is an increase in the number of copies of a specific DNA fragment; can be in vivo or in vitro. In forensic DNA testing laboratories, this refers to the use of the PCR technique to produce many more copies of DNA at specific genetic loci.

**3.3** Capillary electrophoresis is an electrophoretic technique for separating DNA molecules by their relative size based on their differential migration through a capillary tube filled with a liquid polymer.

**3.4** Case-type samples are samples from known individuals with known testing results prepared within the laboratory to simulate a range of samples typically encountered by the testing laboratory in casework. The use of a range of test samples in validation studies facilitates the development of protocols for casework.

**3.5** Critical reagents are chemicals or other materials used in testing whose performance is vital to the success of the test as determined by empirical studies or routine practice.

**3.6** The DNA type derived from mitochondrial DNA is a specific sequence of nucleotides at a given mitochondrial locus. These types may or may not be described in relation to a reference DNA sequence.

**3.7** Genus is a level of taxonomic classification that defines a group of related species.

DRAFT

**3.8** Haplotype is a set of linked DNA variations, or polymorphisms, that tend to be inherited together (e.g. commonly used for mitochondrial or Y-chromosome analysis). A haplotype can refer to a combination of alleles or to a set of single nucleotide polymorphisms (SNPs) found on the same chromosome.

**3.9** Heteroplasmy is the presence of more than one mitochondrial DNA (mtDNA) sequence or type within a single individual.

**3.10** A marker (DNA) is a gene or specific DNA sequence of known location on a chromosome or genome; used as a point of reference in the mapping of other loci.

**3.11** Mitochondrial DNA is a small, circular DNA molecule located in eukaryotic mitochondria that is typically maternally inherited; the resistance to degradation and, presence of multiple copies of mtDNA in each cell makes it useful with samples originating from limited or damaged biological material.

**3.12** Phylogenetic coverage is a criterion used to guide the selection of species for comparative analysis and species determination; the selection of species is based on genetic relatedness.

**3.13** Polymerase chain reaction (PCR) is an enzymatic process by which a specific region of DNA is replicated during repetitive cycles that consist of the following: denaturation of the template; annealing of primers to complementary sequences at an empirically determined temperature; and extension of the bound primers by a DNA polymerase. The goal of the PCR process is to generate many copies (termed products or amplicons) of a specific region of DNA for further analysis.

**3.14** Polymerase is an enzyme that catalyzes the synthesis of nucleic acids on preexisting nucleic acid templates.

**3.15** Polymorphism is the occurrence in a population of two or more alleles or physical traits at a genetic locus. This variation within a population permits the differentiation of individuals via DNA testing or physical traits.

**3.16** Population is a group of organisms of the same species in a defined geographic area, such that any pair of members can interbreed.

**3.17** Primer is a short polynucleotide chain, usually 18–30 bases long, which targets a specific region of the template DNA and allows a DNA polymerase to initiate synthesis of a complementary strand; two primers are required for amplification of a particular section of double-stranded DNA. The primers are complementary to opposite strands and are designed to bracket the region of interest for amplification. Polymerization is initiated at the 3' end of the primer and extends in a 5′ to 3′ manner.

**3.18** Reference materials are biological specimens of known identity or data derived from them.

**3.19** Sanger sequencing is a method of DNA sequencing for determining the order of bases in a DNA molecule based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication. See sequencing below.

DRAFT

**3.20** Sensitivity studies are a set of critical studies performed during developmental and/or internal validation of DNA or other test methods designed to define the lower and upper limits/ bounds of an assay to accurately detect an analyte.

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

**3.22** Species is a level of taxonomic classification that denotes interbreeding natural populations that are reproductively isolated from other interbreeding natural populations.

**3.23** Species specificity is a reference to studies generally performed during developmental validation of forensic testing assays to assess if non-targeted species are detected in an assay (e.g., detection of microbial DNA in a human assay). The detection of non-targeted species does not necessarily invalidate the use of the assay, but may help define the limitations of the assay.

**3.24** Taxonomic authorities are literature references accepted by the relevant scientific community and providing the classification (e.g. family, genus, species) for a group of organisms.

**3.25** Taxonomic identification is the classification of an organism to family, genus, species, etc. These analyses are based on class characters diagnostic for the taxonomic level in question.

**3.26** Validation is the process of performing and evaluating a set of experiments that establish the efficacy, reliability, and limitations of a method, procedure, or modification thereof; establishing recorded documentation that provides a high degree of assurance that a specific process will consistently produce an outcome meeting its predetermined specifications and quality attributes.

**3.27** Variation (interspecific) is the diversity present in a character or set of character states that occurs between different species .

**3.28** Variation (intraspecific) is the diversity present in a character or set of character states that occurs within the same species.

DRAFT

## 4 Standards

**4.1** If it is determined that a particular area listed below is not applicable to the primers/method being validated, an acceptable narrative of the basis of this determination shall be present in validation documentation.

**4.2** If validation reveals that the new marker, in any of the areas addressed below, is not suitable for the intended use, the new marker should not be used, unless an acceptable narrative of the reason for use is presented.

**4.3** The following areas should be addressed in a validation of new primers for amplification and sequencing:

**4.3.1** Characterization of loci: The following characteristics of a genetic region should be determined and documented, including:

a) the location of the genetic region (nuclear or mitochondrial target),

b) the technological basis for identifying the genetic marker (for example, capillary electrophoresis, DNA sequencing, or hybridization assays)

c) a description of any known heteroplasmy and/or cytosine-rich regions.

**4.3.2** Species specificity: The ability to detect information from non-targeted species should be determined, including:

a) species likely to be present in the laboratory (for example, human or species of pets owned by laboratory staff),

b) species likely to be present in combination with the species of interest on evidentiary items (for example, predator DNA mixed with targeted prey DNA),

c) species closely related to the species of interest, to demonstrate appropriate phylogenetic coverage using reference materials following documented taxonomic authorities.

NOTE - The detection of genetic information from non-targeted species does not necessarily invalidate the use of the assay, but may help define the limits of the assay.

**4.3.3** Sensitivity studies: Assess the amplification of a range of DNA quantities to determine the expected results with DNA concentrations at the extreme high and low ends of a concentration gradient applicable to the sample type being tested.

NOTE - Examples of values to be determined include dynamic range, ideal target range, and limit of detection.

**4.3.4** Case-type samples: The ability to obtain reliable results should be evaluated using samples that are representative of those typically encountered by the testing laboratory for the species of interest.

**4.3.5** Population studies: The distribution of polymorphisms within a population found in the genetic region in question should be catalogued:

a) For taxonomic identification applications, an assessment of the degree of intraspecific versus interspecific variation for the targeted genetic region should be performed.

b) For mitochondrial haplotyping applications, a sufficient number of individuals should be sequenced to allow a confidence interval to be reported for any inclusion.

**4.3.6** PCR-based studies:

DRAFT

a) Primer sequences should be readily available. If this is not possible, a clear narrative of explanation should be present in validation documentation.

b) The reaction conditions needed to provide the required degree of specificity and robustness should be evaluated. These include, but are not limited to, thermal cycling parameters, and the concentration of primers, magnesium chloride, DNA polymerase, and other critical reagents.

c) Verify that the target region is amplified with minimal cross amplification of non-target areas and that the amplicon can be reliably sequenced.

DRAFT

## Annex A
(informative)

## Bibliography

This is not meant to be an all-inclusive list as the group recognizes other publications on this subject exist. Additionally, any mention of a particular software tool or vendor as part of this bibliography is purely incidental, and inclusion does not imply endorsement by the authors of this document.


1] ANAB ISO/IEC 17025:2005 - FORENSIC SCIENCE TESTING LABORATORIES Accreditation Requirements. (AR 3028)

2] Hillis, D.M., C. Moritz, and B.K. Mable. 1996. Molecular Systematics, Second Edition. Sinauer Associates, Sunderland, MA.

3] International Organization for Standardization. 2005. ISO/IEC 17025:2005 General Requirements for the Competence of Testing and Calibration Laboratories.

4] Moore, M. K. and Kornfield, I. L. 2012. Best practices in wildlife forensic DNA. In, Wildlife forensics: methods and applications, 1st ed. Edited by J. E. Huffman and J. R. Wallace, pp. 201-236. Chichester: Wiley-Blackwell.

5] Scientific Working Group for Wildlife Forensic Sciences. 2012. SWGWILD Standards and Guidelines v2.0. Available from http://www.wildlifeforensicscience.org/swgwild/.


DRAFT