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Wildlife Forensics Validation Standards—STR Analysis

Wildlife Forensics Validation Standards- STR Analysis

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Foreword

This document addresses the particular need in wildlife forensics to validate new nuclear STR (short tandem repeat) primer sets in laboratories that have previously validated STR analysis methods, and are expanding the primer sets used for source attribution. 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 necessitate the use of different panels of STR markers. These marker panels are not available as commercial "kits", and each panel is applicable only to a single species or small group of closely related species. Laboratories need to be able to validate new STR panels in a straightforward and clearly defined way since this is a regular occurrence. Existing developmental and internal validation documents are all-encompassing, and this further guidance and details are required for laboratories who routinely expand existing panels or develop new panels.

This standard revised, prepared, and finalized by the Wildlife Forensics Consensus Body of the AAFS Standards Board. It 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 AAFS Standards Board. All hyperlinks and web addresses shown in this document are current as the publication date of this standard.

Keywords: *wildlife forensics, validation, DNA, STR analysis, microsatellites*

Abstract: This document provides minimum standards and recommendations for validating new nuclear STR (short tandem repeat) markers for use in wildlife forensic DNA laboratories where the STR genotyping method has already been validated.

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Wildlife Forensics Validation Standards—STR Analysis

1 Scope

This document provides minimum standards and recommendations for validating new nuclear STR (short tandem repeat) markers for use in wildlife forensic DNA laboratories where the STR genotyping method has already been validated.

2 Normative References

There are no normative reference documents, Annex A, Bibliography, contains informative references.

3 Terms and Definitions

For purposes of this document, the following definitions and acronyms apply.

3.1
accuracy
The degree to which the result of a measurement, calculation, or specification conforms to the correct value or a standard.

3.2
amplification
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
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
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
control
A sample of known type, run in parallel with experimental, reference, or evidence samples that is used to demonstrate that a procedure is working correctly.

3.6
critical reagents
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.

3.7
developmental validation
The acquisition of test data and determination of conditions and limitations of a new methodology.

3.8
DNA type
The genetic constitution of an individual at one or more defined locations (also known as loci) in the DNA. A DNA type derived from nuclear DNA typically consists of one or two alleles at several loci (for example, short tandem repeat loci).

3.9
Hardy-Weinberg Equilibrium
A state in which allele and single locus genotype frequencies do not change (on average) from one generation to the next in a population. When alleles in a population are independent, allele and genotype frequencies are related through the Hardy-Weinberg principle: for a locus with 2 alleles P and Q at frequencies of p and q, homozygotes for P are found at frequency p^2, homozygotes for Q are found at a frequency of q^2, and heterozygotes are found at a frequency of 2pq. Use of the theta correction removes the need to assume Hardy-Weinberg equilibrium in the population for which a frequency database is constructed.

3.10
likelihood ratio
The probability of the evidence under one proposition (hypothesis), divided by the probability of the evidence under an alternative, mutually exclusive proposition (hypothesis). The magnitude of its value expresses the weight of the evidence.

3.11
limit-of-detection
1) For a test, the point at which the sensitivity of the test is such that the quantity or concentration of a component present is insufficient to produce a positive test result or be detected.
2) For instrumental analysis, the point below which it is not possible to reliably distinguish analytical signal data from instrument background noise; typically defined as three times the standard deviation of the average background noise; The LOD may be calculated to aid in setting an analytical threshold for the reporting and interpretation of alleles in a DNA profile electropherogram.

3.12
linkage disequilibrium
The nonrandom association, in a population, of alleles at different loci.

3.13
locus
A unique physical location of a gene (or specific sequence of DNA) within a chromosome; the plural of locus is loci (which is pronounced low-sigh).

3.14
internal validation
The accumulation of test data within the laboratory for developing the laboratory standard operating procedures and demonstrating that the established protocols for the technical steps of the test and for data interpretation perform as expected in the laboratory.

3.15
marker (DNA)
A gene or specific DNA sequence of known location on a chromosome; used as a point of reference in the mapping of other loci.

3.16
mixture
DNA typing results originating from two or more individuals.

3.17
mixed DNA sample
Any biological sample containing DNA from more than one individual.

3.18
multiplexing
A test providing for simultaneous amplification of multiple loci.

3.19
nuclear DNA
The DNA contained within a nucleus of eukaryotic organisms comprising the majority of the genome.

3.20
polymerase chain reaction
PCR
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.

3.21
polymerase
An enzyme that catalyzes the synthesis of nucleic acids on preexisting nucleic acid templates.

3.22
polymorphism
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.

3.23
population
A group of organisms of the same species in a defined geographic area, such that any pair of members can interbreed.

3.24
population substructure
The existence of subpopulations with different allele frequencies.

3.25
precision
The degree of mutual agreement among a series of individual measurements, values and/or results.

3.26
primer
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.

3.27
sensitivity studies
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.

3.28
sequencing (DNA)
A laboratory technique used to determine the sequence of bases (A, C, G, and T) in a DNA molecule.

3.29
species
The fundamental unit of taxonomic classification. There is no singular species definition in biology. Essentially the term denotes a group of organisms with a unique shared evolutionary lineage.

3.30
species specificity
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.

3.31
stochastic
Describes 1) chance, or random variation 2) in DNA testing, refers to random sampling error from extracts containing low levels of DNA and/or random variation in selection of alleles amplified at a particular locus.

3.32
stochastic threshold
The peak height value in a DNA electrophoretic profile above which it is reasonable to assume that, at a given locus, allelic drop out of a sister allele in a heterozygous pair has not occurred in a single source DNA sample; due to the possibility of shared alleles in mixed samples, the presence of allele peaks above the stochastic threshold is no guarantee that allele drop out did not occur in mixed DNA sample profiles.

3.33
validation
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.

4 Requirements

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 which includes expanding STR markers and/or multiplexes:

4.3.1 Characterization of genetic markers: The basic characteristics (described below) of a genetic marker should be determined and documented.

a) Inheritance: The mode of transmission of DNA markers from parent to offspring.

b) Detection: Technological basis for identifying the genetic marker (for example, capillary electrophoresis).

c) Polymorphism: Type of variation.

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

a) Species that are likely to be brought into the laboratory (for example, humans, or any species of pets)

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)

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 and stochastic studies: The ability to obtain reliable results from a range of DNA quantities, to include the upper and lower limits of the assay, should be evaluated.

a) The following key values should be determined:

    1) optimal amount of template DNA,

    2) limit-of-detection,

    3) stochastic threshold.

4.3.4 Precision and accuracy of the assay should be demonstrated.

a) Repeatability: Replicates of the same sample amplified by the same operator and/or detection instrument should be evaluated. All replicates should produce the same DNA type.

b) Reproducibility: Replicates of the same sample amplified at another time by different operators and/or detection instruments should be evaluated. All replicates should produce the same DNA type.

4.3.5 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.

4.3.6 Population studies: The distribution of genetic markers in populations should be determined in relevant population groups.

a) Loci should be evaluated for:

    1) linkage disequilibrium,

    2) Hardy–Weinberg Equilibrium.

b) The population should be evaluated for substructure (for example, F_{st} and Θ) so that it may be applied in statistical interpretations.

c) If the markers are intended to be used for source attribution, then a sufficient number of individuals and/or populations must be tested to allow likelihood ratios to be assigned.

4.3.7 Mixture studies: If mixtures are to be analyzed using the new markers, the ability to obtain reliable results from mixed DNA samples should be determined.

4.3.8 PCR-based studies: The following should be available for all relevant species that are being validated in the laboratory.

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) The potential for differential amplification among loci, preferential amplification of alleles at a locus, and stochastic amplification (for example, excessive allelic signal imbalances due to the random sampling and amplification of low template quantities) should be evaluated.

d) The effects of multiplexing should be evaluated.

NOTE For addition of a new primer set to an existing multiplex, this can be a single reaction verifying that the current reaction conditions function for the new marker. For adding a new published species multiplex, this should include a range of values and concentrations to pinpoint the reaction conditions that provide consistent amplification.

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.

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