Wildlife Forensics Validation Standard - Validating New Primers for Sequencing

WHAT IS AN AAFS STANDARD FACTSHEET?

The AAFS produces clear, concise, and easy-to-understand factsheets to summarize the contents of technical and professional forensic science standards on the OSAC Registry. They are not intended to provide an interpretation for any portion of a published standard.

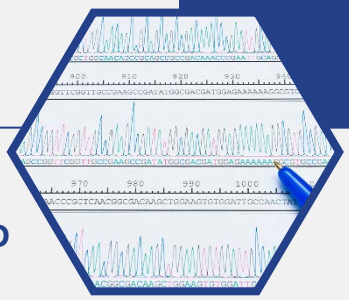
WHAT IS THE PURPOSE OF THIS STANDARD?

Wildlife forensic scientists encounter a variety of species, substrates, and conditions of samples. This standard provides requirements and recommendations for validating new primers for sequencing of DNA from samples submitted to the forensic science service provider (FSSP) when existing primers are not appropriate or are unavailable.

This standard is used in combination with an FSSP's existing, validated DNA sequencing method and with ISO/IEC 17025 for accredited providers.

WHY IS THIS STANDARD IMPORTANT? WHAT ARE ITS BENEFITS?

DNA sequencing is often used by wildlife FSSPs as a tool for taxonomic identification and/or mitochondrial haplotyping for a variety of species. Development and validation of new primers for sequencing may be needed when existing primers are not suitable for a required application. This standard outlines requirements for validating new primers that will be used for the amplification and sequencing of DNA in wildlife forensic applications.



HOW IS THIS STANDARD USED, AND WHAT ARE THE KEY ELEMENTS?

This validation standard is used concurrently with existing primer development, PCR amplification, and DNA sequencing protocols. Key elements of designing and validating new primers are 1) the characterization of loci of interest and 2) the species specificity.

Studies to be undertaken prior to using new sequencing primers in casework include sensitivity studies applicable to the sample type being tested and the use of case-type samples that are representative of those typically encountered.

Requirements for validation of new sequencing primers include PCR-based studies that demonstrate reproducibility, specificity, and robustness. Factors to consider include the availability of primer sequences, PCR amplification conditions, minimal cross amplification of non-target species, and confirmation that the target amplicon can be reliably sequenced.

For use in taxonomic identification applications (e.g., species of origin), this standard recommends that studies be conducted to characterize intraspecific vs. interspecific variability for the targeted genetic region. For mitochondrial haplotyping applications (e.g., population inclusion), this standard recommends that a sufficient number of individuals be sequenced to show the range of polymorphisms within a population, thus allowing a confidence interval to be calculated for any inclusion of an individual in that population.

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