



A138 Developmental Validation of an Improved STR Multiplex for the Forensic Analysis of Canine DNA

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After attending this presentation, attendees will be able to acquaint those in the world of human forensic genetics with the status and potential of canine forensic genotyping. This will be accomplished through the presentation of applied research and forensic casework.

This presentation will impact the forensic community by broadening the understanding of canine forensic DNA testing and to encourage interested laboratories to consider its implementation. Expansion of crime lab capabilities to include canine genotyping has the potential to significantly impact the scope and magnitude of forensic DNA testing services and expand investigational opportunities.

The molecular analysis of biological evidence has revolutionized the criminal justice system. Its power has been used to both incriminate the guilty and free the innocent. While analysis of human DNA has been extensively vetted in courtrooms around the world, forensic analysis of non-human DNA is still gaining acceptance. To promote the admission of animal DNA evidence into the criminal justice system, practitioners must adhere to the same comprehensive validation guidelines that have been established for human DNA evidence. The Scientific Working Group on DNA Analysis Methods (SWGAM) guidelines encourage the publication of validation studies that play an important role in the acceptance of new scientific techniques.

As the oldest domesticated species, dogs (*Canis lupus familiaris*) inhabit 39% of households in America and, after humans, are the species of greatest forensic interest. The forensic analysis of dog DNA has been the subject of several published case reports, but there have been no peer-reviewed published validation studies on the marker panels used. Numerous genetic markers have been successfully employed to individualize canids for parentage verification, breed identification, phylogeny, and diversity assessment; however, due to the quality and quantity of DNA often encountered in forensic samples, forensic analysis requires the application of more stringent marker selection criteria. To address the lack of a standardized and validated canid forensic panel that meets those criteria, a unique opportunity was exploited to mine the recently published 7x dog genome sequence data (Broad Institute, CanFam 2.0). Publicly available markers were first masked to eliminate them from consideration. Tools were developed to query the genome for GAAA and GATA repeat motifs of 10-25 repeat lengths to identify novel tetra-nucleotide repeat markers. Over 2000 potential markers covering all thirty-eight domestic dog autosomes were identified. Candidate markers were screened for heterozygosity, polymorphic information content (PIC), probability of exclusion (PE), and ease of scoring. Fifteen unlinked highly polymorphic tetranucleotide-repeat markers were identified and assembled with the SRY sex-determination marker into a multiplex capable of generating a full DNA profile with less than 0.1 ng of nuclear DNA. To demonstrate the accuracy, precision, and reproducibility of the test, validation was carried out according to the revised SWGAM guidelines for developmental validation. Mutation rates of 0% to 0.82% were assessed through genotyping of the expanded canine Cornell Reference family. Population statistics were generated from approximately 2500 blood and buccal samples representing both registered purebred dogs and outbred convenience samples. This panel has the potential to be not only a valuable tool for the emerging field of veterinary forensic science but also demonstrates utility for parentage verification in highly inbred dog populations and for the phylogenetic analysis of various canid populations.

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