A139 The Development and Validation of a Canine STR Reagent Kit for Use in Forensic Casework

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After attending this presentation, attendees will be familiar with details related to development and validation of an STR reagent kit for dog and with population data generated using the reagent kit. Dog hair is commonly related to forensic casework but validated kits have so far been unavailable.

This presentation will impact the forensic community by demonstrating large-scale canine population data generated using a new reagent kit developed for forensic casework. Dog hair is commonly related to forensic casework but validated STR kits have been unavailable. Therefore, this type of potentially important evidence has been underutilized by the community. The population data will be freely available through NIST website for the forensic research community.

Biological material from pet dogs remains a largely untapped evidentiary resource in forensic investigations. The lack of well-defined STR loci, validated canine PCR/STR kits, a standardized and publically accessible database, and a well-developed nomenclature have contributed to this under-utilization.

To promote the use of domesticated dog-derived evidence, a reagent kit was developed, that enables multiplex PCR amplification of 18 STRs, and the canine sex determining Zinc Finger marker. Validation studies assessing the robustness and reliability of the reagent kit in forensic DNA typing included sensitivity testing, reproducibility studies, intra- and inter-locus color balance studies, peak height ratio determination, characterization of artifacts such as stutter percentages and dye blobs, mixture analyses, species-specificity analyses, and case type sample analyses. The kit robustly amplified domesticated dog samples and consistently generated full 19-locus profiles from as little as 125 pg of dog genomic DNA. In addition, wolf DNA samples could be analyzed with the kit.

The reagent kit was also used in a U.S. domesticated dog population study, focussing on the capability of the multiplex in generating genetic data that sufficiently assessed the informativeness of canine forensic DNA profiles. A database comprising the raw and binned genotype data generated using this reagent kit has been uploaded onto the National Institute of Justice website (STRBase). Allele frequencies were estimated for 236 unrelated, pedigreed, and 431 mixed breed dogs residing in the
U.S. Average random match probability was 1 in 2 X 10^{33}, using the regional database, and 1 in 4 X 10^{39} using the breed dataset. Genetic diversity among and within both datasets were compared. Each pedigreed dog population was genetically distinct and could be differentiated from the mixed breed dog population. The results herein provide further support for using the allele frequency data with the canine STR reagent kit to convey the significance of identity testing for forensic casework, parentage testing, and dog breed assignment.

A nomenclature for canine-specific markers included in the reagent kit was developed based on internationally recognized recommendations for human forensic STR loci. Representative alleles were sequenced from each of the 18 STRs and the sex-typing marker included in the kit. The sequence information from the STR alleles was used to create an operationally based nomenclature. The sequence data also reflects the impact of point mutations, insertions and deletions within and outside the core repeat structures of the loci. Structural variation can affect the mobility of fragment sizing based on capillary electrophoresis, possibly confounding allele calls, allele numbers, and estimates that rely on allele frequency. Because an understanding of the STRs' sequence structure will improve the accuracy and precision of allele fragment sizing in U.S. canine forensic genetic testing, the expected allele sizes were calculated and their repeat stuctures defined based on novel allelic sequence information. Thus, it is recommend the use of an established nomenclature system.

Canine, STR, Validation

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