A156 FidoTyping™ and FidoSearch™: Validation of a Forensic Canine Mitochondrial DNA Protocol and a New On-Line Canid Mitochondrial Hypervariable Region Database

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After attending this presentation, attendees will be aware of laboratory protocols and a new database for the forensic use of mitochondrial DNA (mtDNA) analysis in dogs and other canids.

This presentation will impact the forensic community by demonstrating that mtDNA analysis of domestic dog hair is straightforward and that the current inability to place matches between crime scene hairs and donor dogs in a population genetics or statistical context for meaningful presentation in court can be resolved by use of a new validated forensic database.

Canine hairs may link suspects, victims, or crime scenes. MtDNA analysis applied with few modifications from human mtDNA protocols can provide nucleotide sequence data from the canine hypervariable region for comparisons between samples. As in other species, a published and curated reference dog sequence is available as a benchmark (Genbank, NC_002008).[2] In canids, the mtDNA hypervariable region extends from nucleotide positions (nps) 15,458-16,727, and contains 30 copies of a 10 bp imperfect repeat region (at nps 16130-16430); the tandem repeats are difficult to analyze and relatively uninformative. For a standard forensic analysis, overlapping hypervariable regions HV1 (nps 15431-15782, 352 bp), HV2 (nps 15739-16092, 354 bp), and HV3 (nps 16451-00014, 298 bp)[3] are amplified and sequenced. Abundant canid mtDNA sequences (N = 2,807, including 866 and 696 domestic dogs from Europe and North America, respectively) were available in Genbank to populate the new forensic database, where standardization of the database entries was per.[4] To aid in developing a statistical forensic context for canine mtDNA matches, a software package called FidoSearch™ was developed.

For laboratory validation of the analytical process (FidoTyping™), a total of 18 domestic dogs and wolves were sampled for various tissues (guard and undercoat hairs, saliva, blood) to establish the optimum conditions for canid mtDNA analysis. Animals tested more than once gave the same profile for each analysis; a positive control DNA was prepared for future use in casework. No substantive modifications to standard Mitotyping protocols (extraction, amplification, or sequencing) were necessary to analyze the mitochondrial DNA of dogs. New primer pairs based on[3] amplify and sequence with reliability providing a robust product. Pale and fine undercoat hairs down to 0.5 cm in size amplified well. Some cross-species primer hybridization was observed between dog, cat and human in the limited number of experiments performed, and

as it is impossible to test all mammalian species to check for cross- hybridization, all amplification products that result from casework will be need to be sequenced and submitted to Genbank for a species identification as a confirmation of canid origin.

The validation of the database and its accompanying search software package, FidoSearch™, will be described in the presentation. This database and search engine will be resident and searchable on the Mitotyping Technologies website (www.mitotyping.com). The software package will allow entry of a profile of interest, designation of species (*Canis familiaris*, *C. lupus*; *C. latrans*; etc), continent of interest, setting of search parameters, and printout of final results.   Partial or full hypervariable region profiles may be entered and polymorphisms will be entered according to standard parameters, allowing for ambiguous sites, insertions, and deletions, as well as all polymorphic variants. **References:**

[1]   Pereira L, Van Asch BA, Amorim A. Standardisation of nomenclature for dog mtDNA D-loop: A prerequisite for launching a *Canis familiaris* database. Forensic Science International 2004 141: 99-108.

[2]   Kim KS, Lee SE, Jeong HW, Ha JH. The complete nucleotide sequence of the domestic dog (*Canis familiaris*) mitochondrial genome. Molecular Phylogenetics and Evolution 1998 10: 210-220.

[3]   Gundry RL, Allard MW, Moretti TR, Honeycutt RL, Wilson MR, Monson K et al. Mitochondrial DNA analysis of the domestic dog: Control region variation within and among breeds. Journal of Forensic Sciences 2007 52: 562-572.

[4]   Pereira L, Freitas F, Fernandes V, Pereira JB, Costa MD, Costa S et al. The diversity present in 5140 human mitochondrial genomes. American Journal of Human Genetics 2009 84: 628-640.

Canine, mtDNA, Database