Forensics Team 2

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Date: 14 December 2010

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Case: Death of Professor Millstone

The objective of this case is to use nuclear and mitochondrial DNA, mtDNA, analysis to determine the identity of Professor Millstone's killer. Suspects include Professor Hedd and a disgruntled student. Canine hair and saliva were taken from wounds on the victim found at the crime scene, from Professor Hedd's female dog, the student's male dog, and known control male and female dogs. DNA was extracted from hair and saliva using Tissue and Hair Extraction Kits and DNA IQ Kit from Promega. Specific loci of the nuclear DNA, PEZ 2, PEZ 15 and VWF.X, were amplified using the Polymerase Chain Reaction, or PCR[1,2]. It was possible to do this analysis at these specific loci due to regions of DNA called short tandem repeats, or STR, which are simple sequences that are hypervariable and appear a different number of times in different dogs. Comparison of a number of these regions will yield a positive match between the samples found on the body of Professor Millstone and the samples taken from the suspects' dogs. In addition, a tandem repeat region in the control region of canine mitochondrial DNA not present in the DNA of humans offers the opportunity of identificaton from mitrochondrial DNA and sex chromosomes were amplified using specific canine primers. WD3 and WD6 for the primers used for amplification of mtDNA[3,4]. The primers for all PCR amplification were the reverse compliment sequences at the boarders of the STR regions to be amplified for identification with gel electrophorisis. These amplified DNA sequences were run on either 6% polyacrylamide gels, PAGE gels stained with SYBR Gold stain, or 1.5% agarose gel, which were stained and examined with ethidium bromide. The resultant bands were compared to the exACT Gene 50 bp gene DNA ladder from Fisher Scientific. After measuring significant bands, plots of the logs of estimated base pairs were generated.

After observing PEZ2 bands (Figure 2), it was determined that they provided insufficient data to aid in our analysis due to the incomplete nature of the results, even when combining data from Tuesday and Thursday lab sections. Mitochondrial DNA samples obtained from the victim and two suspects indicated that the dog present at the crime scene, as well as the dogs belonging to both suspects are all of the same maternal line (Figure 3). The collected evidence was compared to DNA obtained and amplified from both male and female control dogs. This is in keeping with the fact that both suspects' dogs came from the same breeder. The VWF.X samples provide similar number of base pairs among all dogs, which increases probability that the dogs of the suspects are immediately related (Figure 1). The PEZ 15 samples, though not fully complete in hair and saliva, reinforce the conclusion from the VWF.X samples that the dogs of the suspects share the same maternal lineage and paternal lineage. Gender analysis of the samples provides inconclusive and inconsistent data compared to the known genders of the suspects' dogs (Figure 4). These samples were compared to control male and female DNA. Issues calling into question the validity of the sex determination gel came when it was determined that the know genders of the suspects' dogs does not match with the genders determined by DNA typing. Professor Hedd owns a female dog, but analysis of the saliva of this dog indicates that it is a male. This result calls into question all of the gender determination done with these dogs, and thus this step of the investigation must be repeated.

Findings from mtDNA analysis do support a constant profile independent of tissue type. There is a probability of 7.1% for two mtDNA fragments from random canines to match in the way the mtDNA fragments matched in samples between suspects and the crime scene. mtDNA analysis does not exclude any of the suspects from further investigation. For the VWF.X nuclear locus, the probability that two unrelated canines would share the same number of short tandem repeats as both suspects and evidence do, is 0.319. For the PEZ 15 nuclear locus, the probability that two unrelated canines would share the same number of short tandem repeats as both suspects and evidence do, is 0.043. The probability that two that two unrelated canines would share the same bands in mtDNA and nuclear DNA analysis as both suspects and evidence do, is 0.0979%. This figure indicates the sequence is specific to one dog in 1021 dalmations. This final calculated probability certainly implicates the two suspects, but it does not conclusively determine the identity of the killer.

Evidence not only does not single out a suspect but also raises questions which further investigation must address. One issue that arises is that the two suspects' dogs are clearly closely related, so there must be additional checks with the breeder to determine positively how the dogs in question are related to each other. The gender determination typing must be repeated, and it is possible that the evidence samples from the dogs of the suspects have been contaminated, thus it would be in the best interest of justice to collect these samples again. Due to the fact that the dogs of the suspects are known to be of different genders, it is highly likely that gender analysis will provide the best opportunity for solving this case. Furthermore, repeating all gel electrophoresis order to generate usable data from the Pez2 STR region. An additional point of comparison will decrease the probability that anyone but our suspects could have committed this crime. In addition, other dogs bred from the same two parental lines must be investigated to ensure no other suspects in this case.

Inconsistent results from all canine samples, specifically from the gender analysis, suggests that samples from the crime scene and from suspects' dogs must be recollected.

Reference:

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3) Gundry, R. L., Allard, M. W., Moretti, T. R., Honeycutt, R. L., Wilson, M. R., Monson, K. L., and Foran, D. R. (2007) Mitochondrial DNA analysis of the domestic dog: control region variation within and among breeds, *J. Forensic Sci. 52*, 562-572.

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