Forensics Team 1

To: Homicide Squad

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Re: Professor Millstone Murder Case

Using the provided samples this laboratory sought to identify the dog responsible for the murder of Dr. Millstone. After careful analysis of the chromosomal and mitochondrial DNA from dog hair and dog saliva samples submitted, the results obtained do not support a definitive conclusion as to the identity of the attacker based on the canine evidence. However, the results do exonerate Dr. Hedd and indicate that the canine at the scene of the crime must be a closely related dog, possibly the dog of the suspect student.

Evidence submitted included dog hairs and saliva swabs from the victim, and the dogs of both suspects, Dr. Hedd and an unidentified student, and control male and female samples. Isolation and amplification of the hair and saliva samples was performed using Promega Tissue and Hair Extraction Kit and the DNA IQ kit, and PCR respectively. The extracted DNA was used as template for the polymerase chain reaction (PCR) amplification of PEZ2, PEZ15, and VWF.X, chromosomal short tandem repeat (STR) Regions, CHR.X and SRY, sex specific markers, and W3/6 , a mitochondrial STR locus (1, 2). The amplification of the sex specific markers produces a fragment of a length that is specific to either the X or Y chromosome allowing the sex of the canine to be determined. STR regions are regions of highly variable repeating motifs and can therefore be used in order to identify individuals. These are all canine specific primer sets, which eliminates the possibility of human contamination as a source of error. The PCR products were analyzed by either polyacrylamide gel electrophoresis (PAGE) or agarose gel electrophoresis and the canine STR and sex profiles were determined.

Figures 1 and 2 show the PAGE gel analysis of the nuclear PCR-STR products. Generally, the Millstone, Hedd and student samples had identical STR profiles. Both the number of bands and the calculated size of the bands were identical (Table 1). Unfortunately, there was an issue with the amplification of the student hair and saliva samples for PEZ2, for all of the hair samples for PEZ 15, and the student hair for VWF.X. In all cases, except for student samples for PEZ2 (which was also not present in the back up gel run by the other lab), there is still applicable data showing the comparisons across the three samples. This is most likely a PCR failure since the mitochondrial amplification of the same extracted DNA sample was successful. The calculated probability of a randomly selected canine having this profile is .200%. As was stated in the investigators report, both suspect dogs were bought from the same breeder. Based on this information, and obtained STR profiles from the gel, there is reason to believe that all three samples were taken from closely related dogs, likely as close as siblings, or even twins. From this it can be concluded that the dog that attacked Professor Millstone is either one of the two suspect dogs, or almost certainly a very close relative of those two dogs.

Figure 3 shows the PAGE analysis of the mitochondrial products. The successful amplification of the controls and the ladder indicate that both the PCR and the PAGE analysis were successful. The STR profile obtained for all samples was identical and distinct from the control in the sizes of the PCR products. This profile is identical in both number and length of amplified fragments across all samples tested, regardless of tissue. This does contradict the results of some investigators, however, since only two tissue types were analyzed it is possible that additional analysis would have revealed variation within each dog (3). This indicates that the dogs either all have the same mother, or their mothers are closely related because mitochondrial DNA is only inherited through the female line. The probability of a random canine having this profile is 7.1%.

Figure 4 shows the agarose gel of the tandem PCR amplification of sex specific loci, CHR.X and SRY. The ladder indicates that the gel was run successfully, and the control male and female samples show that the PCR ran successfully and show the banding that is characteristic of the X and Y chromosomes. As shown in Table 1, the presence of the X chromosome is associated with a PCR product of approximately 169bp, and the Y chromosome is associated with a product of approximately 120bp. The investigators' report indicated that the student's dog was a male, and Hedd's dog was a female. The PCR results for the student's and Hedd's dog were equivocal and do not show consistent results for each sample. However, the gel does show that the DNA found on Millstone came from a male canine, suggesting that Hedd's dog was not present at the scene of the crime. The labs from both gels had many anomalies so the results are not conclusive, however, since the control samples did run successfully the data is not completely useless. We suspect that the error was caused by sample contamination. Reports indicate that the sampling may have been done without proper forensic techniques, so new samples should be taken to guarantee proper results.

The complete STR profiles obtained for both the mitochondrial locus and chromosomal loci indicate that the felonious runt in question was either one of the two suspect dogs or a very closely related dog. The probability of a random unrelated dog having the profile found is .014%, which is about 1 out of 7143 dogs. The sex specific PCR indicates that the dog is most likely a male, eliminating Hedd's dog. It is also important to look into the breeding practices of the breeder from which these dogs were bought. If they engaged in interbreeding the number of closely related dogs, which could have provided the profile found, could be quite high. The sampling of dogs closely related to the suspect dogs from the same breeder would assist in the level of confidence of our results. Further investigation should include resampling using proper techniques such as gloves, and use greater care in obtaining the bulb of the hair rather than the shaft running additional loci because for each additional loci

genotyped the probability of another dog having the same STR profile is reduced, and questioning of the breeder to determine the likely number of closely related dogs that could have provided the profile found. In order to definitively identify the dog present at the crime, a successful sex-typing of the samples would be needed.

Gel Figures.docx

References

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