Population genetic study of 10 short tandem repeat loci from 600 domestic dogs in Korea

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Dogs have long shared close relationships with many humans. Due to the large number of dogs in human populations, they are often involved in crimes. Occasionally, canine biological evidence such as saliva, bloodstains and hairs can be found at crime scenes. Accordingly, canine DNA can be used as forensic evidence. The use of short tandem repeat (STR) loci from biological evidence is valuable for forensic investigations. In Korea, canine STR profiling-related crimes are being successfully analyzed, leading to diverse crimes such as animal cruelty, dog-attacks, murder, robbery, and missing and abandoned dogs being solved. However, the probability of random DNA profile matches cannot be analyzed because of a lack of canine STR data. Therefore, in this study, 10 STR loci were analyzed in 600 dogs in Korea (344 dogs belonging to 30 different purebreds and 256 crossbred dogs) to estimate canine forensic genetic parameters. Among purebred dogs, a separate statistical analysis was conducted for five major subgroups, 97 Maltese, 47 Poodles, 31 Shih Tzus, 32 Yorkshire Terriers, and 25 Pomeranians. Allele frequencies, expected (Hexp) and observed heterozygosity (Hobs), fixation index (F), probability of identity (P(ID)), probability of sibling identity (P(ID)sib) and probability of exclusion (PE) were then calculated. The Hexp values ranged from 0.901 (PEZ12) to 0.634 (FHC2079), while the P(ID)sib values were between 0.481 (FHC2079) and 0.304 (PEZ12) and the P(ID)sib was about \(3.35 \times 10^{-5}\) for the combination of all 10 loci. The results presented herein will strengthen the value of canine DNA to solving dog-related crimes.

Keywords: allele frequency, canine short tandem repeat population, individual identification, short tandem repeat

Introduction

Dogs are one of the oldest and most intimate companion animals for humans in Korea. According to the Korean Pet Association, over five million dogs live in Korea and 10 million households own one or more pet animals. Because dogs live close to humans, they are often involved in forensic cases such as dog attacks, murder, animal abuse, missing dogs and robbery [6]. As a result, canine biological evidence found at crime scenes could become essential in solving criminal cases [4,10]. Short tandem repeats (STRs) analysis is a useful tool in forensic identification [4,13]. In cases in which there are genetic matches between known and unknown samples, STR profiling can play an important role in evaluating the value of the match [1,5,7]. Despite the importance of canine STR profiles at the crime scene, canine DNA evidence is still underestimated in forensic investigations [3,17]. However, several countries including Austria (2005), the United States (2009), Hungary (2011), and the United Kingdom (2012) have started constructing canine STR databases [8,12,14,18]. In Korea, canine STR profiling related crimes have been successfully analyzed, resulting in various crimes including animal cruelty, dog-attacks, murder, robbery, and missing and abandoned dogs being solved. However, the probability of a random DNA profile match cannot be calculated because of the lack of canine STR allele data for the dog population.

In Korea, media-inspired copycat crimes of animal cruelty are on the rise. As a result, the demand for establishing related...
laws is rapidly increasing. In addition, dog-bite injuries can be fatal, especially among the young and elderly, and serious injuries can impact people for their entire lifetime. Moreover, the number of abandoned dogs is rapidly increasing, which has resulted in problems such as dealing with abandoned dogs.

Currently, evidence from dog related crime cases can be admitted for trial in Korea. To improve the reliability of canine STR analysis, we determined the allele frequency and forensic informative values from 600 purebred and crossbred dogs [11]. The results revealed that 10 canine STR markers could be suitable for individual identification in forensic cases and enable estimation of the matching probability and discrimination of forensic work.

Materials and Methods

Population

A total of 600 dogs were evaluated to investigate genetic variation in dogs in Korea. Of these, 344 dogs were of 30 different pure breeds and 256 were crossbred dogs. Among the purebred dogs, a separate statistical analysis of five major subgroups (97 Maltese, 47 Poodles, 31 Shih Tzus, 32 Yorkshire Terriers, 25 Pomeranians) was carried to evaluate intra-breed variation.

DNA extraction

Samples were obtained from local animal clinics and the Veterinary Medical Teaching Hospital at Jeju National University in Korea. DNA was extracted from buccal swabs and blood samples using a QIAamp DNA Micro Kit (Qiagen, Germany). All samples from animal clinics were obtained after getting the owners’ consent for DNA analysis. Extracted DNA was quantified on agarose gel. The quantity of DNA was set to 100 ng/μL compared to K562 concentration by serial dilution (40 ng/μL, 20 ng/μL, 10 ng/μL, 5 ng/μL and 2.5 ng/μL). A multiplex kit was optimized for 10 ng/μL to 100 ng/μL DNA.

PCR amplification and quality control

The 10 STR loci (PEZ1, FHC2054, FHC2010, PEZ5, PEZ20, PEZ12, PEZ3, PEZ6, PEZ8, and FHC2079) were amplified in a GeneAmp PCR System 9700 (Applied Biosystems, USA) using the StockMarks Dog Genotyping Kit according to the manufacturer’s instructions. 10 STR markers were co-amplified in the PCR multiplex reaction. The total volume was 10 μL including StockMarks PCR Buffer 1.4 μL, 25 mM MgCl2 0.36 μL, dNTP mix 2.2 μL, AmpliTaq Gold Polymerase 0.36 μL, amplification primer mix 2.8 μL, deionized water 1.9 μL, and 1 μL of template DNA. The amplification process comprised an initial denaturation step at 95°C for 10min followed by 20 cycles of 95°C for 30 sec, 58°C for 30 sec and extension at 72°C for 60 sec, 15 cycles of 95°C for 30 sec, 56°C for 30 sec and extension at 72°C for 60 sec and then final extension at 72°C for 30 min. A multiplex kit was optimized for 10 ng/μL to 100 ng/μL DNA. Canine control DNA (included in the StockMarks Dog Genotyping Kit) was used as a positive control and deionized water as a negative control.

Typing and analysis of data

Capillary electrophoresis was performed on an ABI 3500 Genetic Analyzer (Applied Biosystems) using POP 7, 50 cm capillary arrays and default instrument settings (all Applied Biosystems). The injection parameters on the ABI 3500 were 1.6 kV and 8 sec. Data were analyzed using the GeneMapper ver. 1.2 software. The fragment sizes of the allelic ladder were measured using GeneScan 500 ROX internal sizing standards. An allelic ladder is not provided with the StockMarks Dog Genotyping Kit. Therefore, profiles from positive control canine DNA included with the kit were used to offset the allele bins instead. The positive control provided with the StockMarks Dog Genotyping Kit was used to calibrate the allele sizes. PCR amplification of the canine control DNA template of electrophoresis conducted at different times determined the reproducibility and precision of the data. The dog parentage test and analysis of low copy number of DNA template were successfully resolved with the StockMarks Dog Genotyping Kit. Occasionally, intermediate sized loci were formed due to imperfect repeat tandems. However, the genotyping sizes were calculated automatically using the GeneMapper software based on size estimation of the fluorescent labeled DNA fragments. Fragment sizes were also compared with previous data [9], and the allelic frequencies of each marker were calculated using the GenAIEx 6 software [15].

The data were analyzed using GeneMapper ver. 1.2 with detection of peak amplitude thresholds set to 100 relative fluorescence units (RFUs). For peak quality, the homozygous min peak height was 200 and the heterozygous min peak height was 100. The Hardy-Weinberg equilibrium, estimated coefficients of inbreeding (FIS) within breeds, fixation indices (FIS) among breeds, and total inbreeding (FIT) were determined using an analysis of variance framework implemented by the GenAIEx 6.5 software.

Results

Population genetic statistics parameters, such as allele frequencies, expected (Hexp) and observed heterozygosity (Hobs), fixation index (F), probability of identity (P(ID)), probability of sibling identity (P(ID)sib) and probability of exclusion (PE) were determined using the GenAIEx 6.5 software. The allele frequencies for each marker were calculated using the GenAIEx 6.5 software [15].

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Results

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Heterozygosity values were used to estimate the allele diversity or variation of forensic markers. The expected heterozygosity (Hexp) values ranged from 0.901 (PEZ12) to
Table 1. Allele frequency and forensically informative statistical values of the 10 analyzed STR loci in the "All breeds" samples

| ALLELE | PEZ1 | PEZ2054 | FHC2010 | PEZ5 | PEZ20 | PEZ12 | PEZ3 | PEZ6 | PEZ8 | FHC2079 |
|--------|------|---------|---------|------|-------|-------|------|------|------|---------|
| 96     |      |         |         |      |       |       |      |      |      |         |
| 98     |      |         |         |      |       |       |      |      |      |         |
| 100    |      |         |         |      |       |       |      |      |      |         |
| 102    | 0.024|         |         | 0.507|       |       |      |      |      |         |
| 103    |      |         |         | 0.003|       |       |      |      |      |         |
| 104    | 0.001|         |         |      |       |       |      |      |      |         |
| 106    | 0.084|         | 0.164   |   0.005|       |       |      |      |      |         |
| 107    | 0.003|         |         | 0.004|       |       |      |      |      |         |
| 108    | 0.004|         |         |      |       |       |      |      |      |         |
| 109    |      |         |         |      |       |       |      |      |      |         |
| 110    | 0.157|         | 0.185   |   0.002|       |       |      |      |      |         |
| 111    | 0.028|         | 0.011   |   0.001|       |       |      |      |      |         |
| 112    | 0.002|         |         |      |       |       |      |      |      |         |
| 113    |      |         |         |      |       |       |      |      |      |         |
| 114    | 0.231|         | 0.086   |   0.020|       |       |      |      |      |         |
| 115    | 0.008|         | 0.015   |   0.006|       |       |      |      |      |         |
| 117    | 0.001|         |         |      |       | 0.019 |      |      |      |         |
| 118    | 0.219|         |         |      |       | 0.260 |      |      |      |         |
| 119    | 0.038|         |         |      |       | 0.284 |      |      |      |         |
| 120    |      |         |         |      |       | 0.036 |      |      |      |         |
| 121    |      |         |         |      |       | 0.026 |      |      |      |         |
| 122    | 0.052|         |         |      |       | 0.001 |      |      |      |         |
| 123    | 0.026|         |         |      |       | 0.043 |      |      |      |         |
| 124    |      |         |         |      |       |       |      |      |      |         |
| 125    | 0.001|         |         |      |       |       |      |      |      |         |
| 126    | 0.033|         |         |      |       |       |      |      |      |         |
| 127    | 0.063|         |         |      |       |       |      |      |      |         |
| 128    |      |         |         |      |       | 0.023 |      |      |      |         |
| 129    |      |         |         |      |       | 0.023 |      |      |      |         |
| 130    | 0.001|         |         |      |       | 0.039 |      |      |      |         |
| 131    | 0.024|         |         |      |       |       |      |      |      |         |
| 132    |      |         |         |      |       | 0.005 |      |      |      |         |
| 133    |      |         |         |      |       | 0.033 |      |      |      |         |
| 134    |      |         |         |      |       | 0.002 |      |      |      |         |
| 135    | 0.001|         |         |      |       | 0.003 |      |      |      |         |
| 136    |      |         |         |      |       | 0.005 |      |      |      |         |
| 139    |      |         |         |      |       | 0.001 |      |      |      |         |
| 140    |      |         |         |      |       | 0.003 |      |      |      |         |
| 143    |      |         |         |      |       | 0.002 |      |      |      |         |
| 144    |      |         |         |      |       | 0.001 |      |      |      |         |
| 146    |      | 0.057   |         |      |       |       |      |      |      |         |
| 149    |      |         |         |      |       | 0.001 |      |      |      |         |
| 150    |      | 0.169   |         |      |       |       |      |      |      |         |
| 151    |      | 0.047   |         |      |       |       |      |      |      |         |
| 153    |      |         |         |      |       | 0.003 |      |      |      |         |
| 154    |      | 0.077   |         |      |       |       |      |      |      |         |
| 155    |      | 0.136   |         |      |       |       |      |      |      |         |
| 158    |      | 0.005   |         |      |       |       |      |      |      |         |
| 159    |      | 0.077   |         |      |       |       |      |      |      |         |
| 162    |      | 0.001   |         |      |       |       |      |      |      |         |
| ALLELE | PEZ1 | PEZ2054 | FHC2010 | PEZ5 | PEZ20 | PEZ12 | PEZ3 | PEZ6 | PEZ8 | FHC2079 |
|--------|------|---------|---------|------|-------|-------|------|------|------|---------|
| 163    | –    | 0.124   | –       | –    | –     | –     | –    | –    | –    | –       |
| 164    | –    | –       | –       | –    | –     | –     | –    | 0.001 | –    | –       |
| 167    | –    | 0.168   | –       | –    | –     | –     | –    | 0.003 | –    | –       |
| 169    | –    | –       | –       | –    | –     | –     | –    | 0.003 | –    | –       |
| 170    | –    | –       | –       | –    | –     | –     | –    | 0.002 | –    | –       |
| 171    | –    | 0.094   | –       | –    | 0.183 | –     | –    | 0.063 | –    | –       |
| 172    | –    | –       | –       | –    | –     | –     | –    | 0.035 | –    | –       |
| 173    | –    | –       | –       | –    | –     | –     | –    | –    | –    | 0.004   |
| 175    | –    | 0.043   | –       | –    | 0.329 | –     | –    | 0.176 | –    | –       |
| 176    | –    | –       | –       | –    | 0.023 | –     | –    | 0.002 | –    | –       |
| 177    | –    | –       | –       | –    | –     | –     | –    | 0.006 | –    | –       |
| 178    | –    | –       | –       | –    | –     | –     | –    | 0.023 | –    | –       |
| 179    | –    | 0.003   | –       | –    | 0.180 | –     | –    | 0.216 | –    | –       |
| 180    | –    | –       | –       | –    | –     | –     | –    | 0.011 | –    | –       |
| 181    | –    | –       | –       | –    | –     | –     | –    | 0.002 | –    | –       |
| 182    | –    | –       | –       | –    | –     | –     | –    | 0.006 | –    | –       |
| 183    | –    | –       | –       | –    | –     | 0.164 | –    | 0.082 | –    | –       |
| 184    | –    | –       | –       | –    | –     | –     | –    | –    | –    | 0.038   |
| 185    | –    | –       | –       | –    | –     | –     | –    | 0.001 | –    | –       |
| 186    | –    | –       | –       | –    | –     | –     | –    | 0.002 | –    | –       |
| 187    | –    | –       | –       | –    | –     | –     | –    | 0.044 | –    | –       |
| 188    | –    | –       | –       | –    | –     | –     | –    | 0.073 | –    | –       |
| 190    | –    | –       | –       | –    | –     | –     | –    | 0.001 | –    | –       |
| 191    | –    | –       | –       | –    | –     | –     | –    | 0.012 | –    | –       |
| 192    | –    | –       | –       | –    | –     | –     | –    | 0.016 | –    | –       |
| 194    | –    | –       | –       | –    | –     | –     | –    | 0.001 | –    | –       |
| 195    | –    | –       | –       | –    | –     | –     | –    | 0.009 | –    | –       |
| 196    | –    | –       | –       | –    | –     | –     | –    | 0.010 | –    | –       |
| 197    | –    | –       | –       | –    | –     | –     | –    | 0.001 | –    | –       |
| 198    | –    | –       | –       | –    | –     | –     | –    | 0.002 | –    | –       |
| 199    | –    | –       | –       | –    | –     | –     | –    | 0.003 | –    | –       |
| 220    | –    | –       | –       | –    | –     | –     | –    | 0.002 | –    | –       |
| 221    | –    | –       | –       | –    | –     | –     | –    | 0.023 | –    | –       |
| 222    | –    | –       | –       | –    | –     | –     | –    | –    | –    | 0.091   |
| 223    | –    | –       | –       | –    | –     | –     | –    | –    | –    | 0.000   |
| 224    | –    | 0.013   | –       | –    | –     | –     | –    | –    | –    | –       |
| 225    | –    | 0.054   | –       | –    | –     | –     | –    | –    | –    | 0.091   |
| 226    | –    | –       | –       | –    | –     | –     | –    | –    | –    | 0.099   |
| 227    | –    | 0.083   | –       | –    | –     | –     | –    | –    | –    | 0.000   |
| 228    | –    | 0.373   | –       | –    | –     | –     | –    | –    | –    | –       |
| 229    | –    | –       | –       | –    | –     | –     | –    | –    | –    | 0.088   |
| 230    | –    | –       | –       | –    | –     | –     | –    | 0.067 | –    | –       |
| 231    | –    | –       | –       | –    | –     | –     | –    | 0.153 | –    | –       |
| 232    | –    | –       | –       | –    | –     | –     | –    | 0.137 | –    | –       |
| 233    | –    | –       | –       | –    | –     | –     | –    | 0.137 | –    | –       |
| 234    | –    | –       | –       | –    | –     | –     | –    | 0.038 | –    | –       |
| 235    | –    | –       | –       | –    | –     | –     | –    | –    | –    | 0.001   |
| 236    | –    | –       | –       | –    | –     | –     | –    | –    | –    | 0.019   |
| 237    | –    | –       | –       | –    | –     | –     | –    | 0.218 | –    | –       |
| 239    | –    | –       | –       | –    | –     | –     | –    | 0.001 | –    | –       |
| 240    | –    | –       | –       | –    | –     | –     | –    | 0.001 | –    | –       |
0.634 (FHC2079). Of the 10 STR loci, the highest observed heterozygosity (Hobs) value was observed in PEZ12 (0.788) and the lowest in FHC2079 (0.375) loci.

\[ P(\text{ID}_{\text{sib}}) \text{ values were between 0.455 (PEZ5) and 0.304 (PEZ12).} \]

The estimated value of \( P(\text{ID}_{\text{sib}}) \) was approximately \( 3.35 \times 10^{-5} \) for the combination of all 10 loci.

Allele frequency and forensically informative statistical values of the 10 STR from intra-breed analysis of five breeds (Maltese, Poodle, Shih Tzu, Yorkshire Terrier, Pomeranian) were evaluated. The results are shown in Supplementary Table 1.

| ALLELE | PEZ | PEZ2054 | FHC2010 | PEZ5 | PEZ20 | PEZ12 | PEZ3 | PEZ6 | PEZ8 | FHC2079 |
|--------|-----|---------|---------|------|-------|-------|------|------|------|---------|
| 241    |     |         |         |      |       |       |      |      |      |         |
| 242    |     |         |         |      |       |       |      |      |      |         |
| 243    |     |         |         |      |       |       |      |      |      |         |
| 245    |     |         |         |      |       |       |      |      |      |         |
| 246    |     |         |         |      |       |       |      |      |      |         |
| 249    |     |         |         |      |       |       |      |      |      |         |
| 256    |     |         |         |      |       |       |      |      |      |         |
| 257    |     |         |         |      |       |       |      |      |      |         |
| 260    |     |         |         |      |       |       |      |      |      |         |
| 261    |     |         |         |      |       |       |      |      |      |         |
| 262    |     |         |         |      |       |       |      |      |      |         |
| 264    |     |         |         |      |       |       |      |      |      |         |
| 265    |     |         |         |      |       |       |      |      |      |         |
| 267    |     |         |         |      |       |       |      |      |      |         |
| 268    |     |         |         |      |       |       |      |      |      |         |
| 269    |     |         |         |      |       |       |      |      |      |         |
| 271    |     |         |         |      |       |       |      |      |      |         |
| 273    |     |         |         |      |       |       |      |      |      |         |
| 275    |     |         |         |      |       |       |      |      |      |         |
| 276    |     |         |         |      |       |       |      |      |      |         |
| 277    |     |         |         |      |       |       |      |      |      |         |
| 279    |     |         |         |      |       |       |      |      |      |         |
| 280    |     |         |         |      |       |       |      |      |      |         |
| 282    |     |         |         |      |       |       |      |      |      |         |
| 283    |     |         |         |      |       |       |      |      |      |         |
| 284    |     |         |         |      |       |       |      |      |      |         |
| 285    |     |         |         |      |       |       |      |      |      |         |
| 286    |     |         |         |      |       |       |      |      |      |         |
| 287    |     |         |         |      |       |       |      |      |      |         |
| 289    |     |         |         |      |       |       |      |      |      |         |
| 290    |     |         |         |      |       |       |      |      |      |         |
| 291    |     |         |         |      |       |       |      |      |      |         |
| 292    |     |         |         |      |       |       |      |      |      |         |
| 293    |     |         |         |      |       |       |      |      |      |         |
| 294    |     |         |         |      |       |       |      |      |      |         |
| 295    |     |         |         |      |       |       |      |      |      |         |
| 296    |     |         |         |      |       |       |      |      |      |         |
| 297    |     |         |         |      |       |       |      |      |      |         |
| 298    |     |         |         |      |       |       |      |      |      |         |
| 300    |     |         |         |      |       |       |      |      |      |         |
| 304    |     |         |         |      |       |       |      |      |      |         |
| 305    |     |         |         |      |       |       |      |      |      |         |
| 308    |     |         |         |      |       |       |      |      |      |         |

Table 1. Continued
The relative distances among breeds were examined by calculating the $F_{ST}$ values. All pairwise comparisons among breeds showed significant $F_{ST}$ values ($F_{ST} > 0, p = 0.01$). Genetic diversity was also determined by calculating the level of inbreeding $F_{IS}$. $F_{ST}$, $F_{IS}$ and $F_{IT}$ estimates for each analyzed STR locus among the five Korean dog population samples are shown in Table 2. Most breeds showed deviations from the Hardy-Weinberg equilibrium across loci as indicated by inbreeding coefficients, $F_{IS}$, significantly $> 0$ (Table 2).

The results show that the genetic diversity of 10 canine STR markers is sufficient to be a valuable tool in solving crime scene casework involving dog samples.

### Discussion

DNA profiling of domestic dogs has become powerful evidence in forensics [6]. In Korea, canine STR profiling-related crimes have been successfully analyzed for over 5 years, resulting in various crimes including animal cruelty, dog-attacks, murder, robbery, and missing and abandoned dogs being solved. However, the probability of a random DNA profile match could not be measured because of the lack of canine STR population data, which sometimes caused delays in cases involving dogs.

We conducted a canine population study of 600 unrelated dogs in Korea using 10 canine STR markers. Calculating match
probability is an essential step in estimating the exact power of a DNA match [2]. The probability of identity (PI), an individual identification estimator, is the probability that two individuals have the same genotype of multiple loci in a random population [7]. It is imperative to determine if the calculated $P_{IDsub}$ value is adequate for forensic applications. A low $P_{IDsub}$ value between $1.0 \times 10^{-3}$ and $1.0 \times 10^{-4}$ is considered reliable for individual identification in natural animal populations [16].

The expected heterozygosity (Hexp) values ranged from 0.901 (PEZ12) to 0.634 (FHC2079), while the $P_{IDsub}$ values were between 0.481 (FHC2079) and 0.304 (PEZ12). Additionally, the estimated values of $P_{IDsub}$ were approximately $3.35 \times 10^{-3}$ for the combination of all 10 loci. The allele frequencies and measures of locus informatives (number of alleles, expected heterozygosity, observed heterozygosity, and power of exclusion) of 558 dogs were calculated in the United States and are available in the Zoogen database [10]. Moreover, population parameters such as allele frequencies, Hardy-Weinberg-Equilibrium, expected and observed heterozygosity, fixation index, and PE of 295 dogs samples from Austria and Germany were calculated [2]. In the UK, the genetic diversity of 285 dogs from 13 popular breeds was analyzed [14], while in Hungary, the allele frequencies of 10 STRs in 668 dogs were determined [18].

Compare to other countries’ dogs database study, the genetic parameters (Hexp, Hob, F, $P_{ID}$, $P_{IDsub}$, and PE) and allele frequency values obtained in this study were very similar to those of other countries based on recently developed canine STR databases.

The purpose of canine STR genotyping is to identify individual dogs. In forensic cases, estimation of population genetic parameters, including allele frequency, is used to differentiate each individual as they can strengthen the weight of canine STR evidence.

Problems related to dogs such as animal cruelty, attacks on people or animals, involvement at the crime scene, property damage and identifying lost pets have been rapidly increasing. In these kinds of crimes, a canine STR profiling-based database for Korea will be a valuable tool during investigation of crimes. In these kinds of crimes, a canine STR profiling-based database for Korea will be a valuable tool during investigation of crimes.

In addition, the data could be a practical alternative to use of a dog microchip registration system to solve missing or abandoned dog cases.

There has been no approach to measure canine genetic parameters of Korean domestic dogs to date. Therefore, these data can provide accurate estimates for forensic informative parameters of dog DNA in Korea. Nevertheless, construction of a canine STR population database in Korea will be a challenge to canine forensic applications.

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**Conflict of Interest**

There is no conflict of interest.

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