Use of the Genome Profiling Method for the Identification of Saliva and Sweat Samples

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The identification of body fluids at crime scenes provides critical evidence that can be used to prove the occurrence of a crime. In this study, the genome profiling (GP) method was utilized to identify saliva and sweat. We randomly amplified cDNA obtained through the RT–PCR approach using RNA samples extracted from saliva and sweat with four different random primers, and performed temperature gradient gel electrophoresis between 15–65°C. The Sp–2 primer was the only primer which generated the species identification dots (spiddos) in all body fluids. The numbers of spiddos found were 11.6 ± 0.89 in saliva and 3.0 ± 1.73 in sweat, and the body fluid type specific spiddos were obtained from electrophoresed gel. Along with previously reported data of semen and vaginal fluid, it was indicated that the GP method might distinguish those four kinds of body fluids. This novel assay is a simple and economical method. Therefore it may be an effective tool for the identification of body fluids at crime scenes. However, further detailed studies, including sensitivity, reproducibility, environmental effect and the effectiveness of detection from mixed stains are necessary before actual forensic investigations.

Key words: RNA, Saliva, Sweat, Genome profiling

Introduction
The identification of various body fluids left at crime scenes is crucial evidence when proving that various crimes have taken place. For instance, it is commonly necessary to establish the presence of saliva on the skin (such as breast or femur) of victims of sexual assault. Additionally, it is necessary to distinguish sweat from saliva, semen and vaginal fluid as sweat is produced on the body surface. At present, the detection of amylase, which is secreted in the salivary glands, is commonly used to establish the presence of saliva. However, recently it has been reported that the detection of statherin and histatin can be useful in saliva identification¹⁻⁵. On the other hand, sweat is regarded as one of the most difficult body fluids to identify.
However, the detection of sweat is also important. For instance, it is important to prove the presence of sweat from the fibers of rope used during strangulation. It can be putatively identified by the presence of urea and lactic acid, E-urocanic acid and L-tyrosine. In 2010, Sakurada et al. reported that the detection of dermcidin through real-time RT-PCR and ELISA can be an effective method for sweat identification.

Recently, a number of new approaches have been developed that compare the mRNA expression levels of specific body fluids and can distinguish various body fluids. Although it is generally believed that mRNA is highly unstable, the stability and specificity of mRNA for identifying body fluids were more effective than expected. Therefore, in the near future mRNA analysis can be a useful tool for body fluid identification. However, methods utilizing mRNA require presently expensive reagents and equipment.

In 2011, we reported the detection of semen and vaginal RNA using the genome profiling (GP) method, a new technique for genomic analysis. Although the GP method was originally designed to identify the profile of genomic DNA, it was established that GP analysis may be utilized not only in genomic DNA profiling but also in cDNA samples that are reverse transcribed from RNA extracted from body fluids. In addition, we also reported that the GP method was utilized in the identification of human blood. As the GP method is simple and relatively cost-effective, it may become a useful novel tool for specific body fluid identification.

In the present study, we applied the GP method to the identification of saliva and sweat. We determined the body fluid type specific species identification dots (spiddos) for saliva and sweat through the GP method. The data from the present study was combined with previously reported data on semen and vaginal fluid, and it is discussed whether this method can be utilized to distinguish saliva, sweat, semen and vaginal fluid prior to human identification using DNA analysis.

**Materials and Methods**

1. **Samples**

   Fresh saliva and sweat samples (samples: n = 5; age 25–45 years) were obtained using procedures approved by the Institutional Review Board of the National Research Institute of Police Science. Total RNA was immediately extracted from a sample (30 μL) of body fluid or the sample was stored at −80°C and RNA was extracted within one month. No RNA stabilization reagent to minimize the degradation of RNA in the samples was used. Total RNA was extracted and reverse transcription was performed as described. The data of semen and vaginal samples analyzed in the previous study was used in the results and discussion of this paper.

2. **Genome profiling (GP) method**

   The GP method was developed in 1991 by Nishigaki et al. This method randomly extracts information regarding the whole genomic arrangement; it is a simple and easily performed technique that can generate abundant information on the extracted DNA. The GP method can be used to classify species of plants, fish, and insects, and to identify mutations. Random PCR was performed on cDNA using one of the following primers: SP–1 (pfm12) (5′-agaacgcgcctg-3′); SP–2 (pfm19) (5′-cagggcgcgcgtac-3′); SP–3 (hunt) (5′-tctgtgctgtgctg-3′); and SP–5 (J) (5′-cagtgctagcat-3′) (first round PCR). Additional PCR using the same primers used in first round PCR to increase sensitivity (second round PCR) was then conducted as described.

   M13 phage and pBR322 DNA were used as the internal reference samples during the GP method. Reference 1 (Ref1; M13 phage DNA) was approximately 200 base pairs (bp), and reference 2 (Ref2; pBR322 DNA) was approximately 900 bp. Amplification was carried out in a PC–320 Thermal Cycler (ASTEC, Fukuoka, Japan). Five microliters of the second round PCR product was mixed with 0.3 μL of Ref1 and Ref2 in a 6× loading buffer double
dye. The mixed sample was loaded on a 6% polyacrylamide gel (μgel). The μgel was set in a thermo gradient gel electrophoresis machine (μTG, TAITEC, Saitama, Japan) with a temperature gradient electrophoresis profile (left 15°C to right 65°C) and electrophoresed at 100 V for 10 min. The electrophoresed μgel was stained using GelRed (Biotium, CA, USA) in a 0.1 M NaCl solution for 10 min. The image from the μgel was captured under UV transillumination on a LAS 4000 Mini (Fujifilm, Tokyo, Japan) and spiddos, which indicate the base sequence specific cleavage points related to the specific temperature and amplified DNA size, were obtained. The gel positions of obtained spiddos were standardized using two reference spiddos (Ref1 and Ref 2) by microTGGE image analysis software.

Results and Discussion

The numbers of spiddos generated utilizing the four different random PCR primers from the body fluid samples are shown in Table 1. Combined with the semen and vaginal fluid data reported previously9, spiddos were successfully generated from all four kinds of body fluids utilizing the SP–2 primer. Spiddos were not generated from all body fluid samples by any of the other three primers. This data indicated that the SP–2 primer was suitable for the detection of these four body fluid types. The SP–2 primer generated between 11–13 spiddos (mean 11.6 ± 0.89) from saliva, 2–6 spiddos (mean 3.0 ± 1.73) from sweat, 2–5 spiddos (mean 3.0 ± 1.22) from semen, and 4–6 spiddos (mean 4.6 ± 0.89) from vaginal fluid. It was possible to gain a similar number of the multiple spiddos from five individual samples in each fluid type. The numbers of the body fluid type specific spiddos in sweat or semen were fewer than those of saliva and vaginal fluid. This may be because the amount of total RNA is not abundantly present in sweat and semen samples or that the RNA extracted from saliva and vaginal fluid also contained RNA from oral and vaginal epithelium. Additionally, it was also reported that about 3000 different types of RNA exist in saliva16. Therefore, it is believed that large amounts of varying RNA types exist in saliva and vaginal fluid. However, it should be pointed out that owing to the difficulty of sample collection only five samples of each body fluid were utilized. It is assumed that the nature of each body fluid type is not always the same. For instance, secretion of vaginal fluid changes with the menstrual period. Therefore, as a result further research is necessary to establish if spiddos can always be obtained from the body fluids tested.

The saliva, sweat, semen, and vaginal fluid sample spiddos profiles were standardized with the reference spiddos. In the GP method, pattern similarity score (PaSS) values are usually
that showed the detection of pathogenic microorganism from the difference in electrophoreosed pattern of random amplification products\textsuperscript{17}. Form our results, the GP method may also be used to identify these body fluids in mixed samples from crime scenes. In addition to the simplicity with which the method is conducted and its cost-effectiveness, new body fluid specific RNA may be found through analyzing spiddos. Before this method can be utilized further studies establishing the sensitivity and reproducibility of this method are necessary. Additionally, the impact of the environment and the effectiveness of the method on dried and mixed stain samples should also be established before use in actual criminal investigation.

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