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The GP Method can be Effective as a Screening Test Before STR Typing

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ABSTRACT The identification of individuals from biological materials found at crime scenes is a critical part of criminal investigations. However, present methods are expensive, complicated, and time consuming. In this paper, the researchers investigated the genome profiling (GP) method as an inexpensive, fast, and simple alternative for obtaining approximate whole genome information from blood, nail, hair bulb, and buccal swab samples. The samples were taken from five individuals to test whether individuals could be identified using the GP method. Each sample group was classified into individual clusters. Despite the small number of the samples, the researchers show that the genome profiling method may be useful for human identification in forensic investigation.

INTRODUCTION

Identifying the origin of biological materials found at crime scenes is central to forensic analysis in criminal investigations. Although this is widely done using the very effective short tandem repeat (STR) analysis, the method is expensive and requires high technical skill, making it difficult to apply in developing countries. In 2000, Nishigaki et al. introduced the genome profiling (GP) method in the field of bioengineering (Nishigaki et al. 2000a), but this method has wider applicability because it enables approximation of whole genome information by random polymerase chain reaction (PCR) and temperature gradient electrophoresis.

Recently, the researchers introduced the GP method into their forensic practice and showed that the method can discriminate body fluid type based on mRNA (Takasaka et al. 2011; Hirata et al. 2013) and can identify human DNA from among animal samples (Suwa et al. 2012). It is also used to detect and discriminate small genomic differences in viral genome (Tanaka et al. 2015). In those studies, the researchers realized that it may also be possible to identify individuals with the GP method. This is important, because the method is inexpensive, fast, and simple, making it an attractive potential alternative method for whole genome analysis when identifying individuals of the same species for forensic purposes.

In this preliminary study, the researchers aimed to investigate whether the GP method can effectively identify human individuals from blood, nail, hair bulb, and buccal swab samples in five individuals. In addition, by analyzing samples taken from the same individual on three different days, the researchers aimed to determine whether sample type could be identified with the GP method. Thus, the researchers evaluated the validity, accuracy, and reliability of this analysis method.

Objective

The objective of this paper is to investigate whether the GP method can effectively identify individuals using human materials.

METHODOLOGY

This study was approved by the Ethical Review Board of Kyoto Prefectural University of

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Medicine (G-98). Written consent was obtained from the five adult volunteers prior to sample collection.

DNA Samples

The researchers collected 100 μL of blood and 20 mg of nail, 20 mg of hair bulb, and buccal swab samples from five volunteers (Nos. 1 to 5). Among the volunteers, the same sets of samples were taken three times over a one-week period, and stored at -70ºC until use. DNA was extracted from the biological material using QIAmp DNA mini kits (QIAGEN, Tokyo, Japan) according to the manufacturer's instructions, with the final DNA concentration set as 1 ng/μL.

GP Method

Random PCR

Samples were randomly amplified with a random primer. The reaction mixture (25 μL) contained 17.5 pmol of SP-1 Primer (pfm12) (5'-agaacgcgcctg-3']) (Biyani and Nishigaki 2003; Nishigaki et al. 2000b), 0.16 mM each of dNTP, 1x ExTaq Buffer, 0.5 units of Ex Taq polymerase (TaKaRa Bio Inc. Shiga. Japan), and 1.0 ng of crude DNA. The reaction mixture was set for the PCR reaction using a PC-320 Thermal Cycler (ASTEC, Fukuoka Japan). After denaturation at 94ºC for 5 min, the researchers performed 30 cycles of PCR at 94ºC for 30 s, at 26ºC for 1 min, and at 47ºC for 1 min, followed by extension at 47ºC for 5 min.

Internal Standard

M13 phage and pBR322 DNA were used as the internal reference samples, according to the GP method (Nishigaki et al. 2000a). Reference 1 (Ref1; M13 phage DNA) was approximately 200 base pairs (bp) and reference 2 (Ref2; pBR322 DNA) was approximately 900 bp. Ref1 underwent PCR amplification using the primers MA1 (5' -tgctacgtctcttccgatgctgtctttcgc-3') and MA2 (5' - ccttgaattctatcggtttatca-3'). The total reaction volume of 50 μL contained 1.0 μg of M13 phage DNA (TaKaRa Bio Inc.), 0.75 U of ExTaq DNA polymerase, 200 μM dNTPs, 0.6 μM of primers, and a PCR buffer supplied by the manufacturer. PCR amplification was performed over 30 cycles at 94ºC for 30 s, 63ºC for 1 min, and 72ºC for 30 s, and finalized at 72ºC for 5 min. PCR amplification of Ref2 was done using the primers Ref6F (5' - gccggcatcaccggcgccacaggtgcggttg-3') and Ref6R (5'-tagcgaggtgccgccggcttccattcaggtc-3'). The total reaction volume of 50 ìL contained 0.25 μg of pBR322 DNA (TaKaRa Bio Inc.), 1.25 U of ExTaq DNA polymerase, 200 ìM dNTPs, 0.7 μM primers, and a PCR buffer supplied by the manufacturer. PCR amplification was performed over 25 cycles at 94ºC for 15 s, 55ºC for 30 s, and 72ºC for 1 min, and finalized at 72ºC for 30 s.

Temperature Gradient Gel Electrophoresis

The two reference DNAs were added to the amplified DNA samples as internal reference standards [molecular weights of 200 bp (ref. 1) and 900 bp (ref. 2)]. Then, 6 μL of the reference DNA was electrophoresed at a temperature gradient of six percent polyacrylamide gel for 10 min at a constant voltage of 100 V using μTG (TAITEC, Saitama, Japan) (Nishigaki et al. 1984, 1992). The temperature gradient was from 15ºC to 65ºC. The electrophoresed gel was then stained in 0.1 M NaCl solution containing 0.03 percent GelRed (Biotium Inc. CA,USA) for 10 min, and a picture of the gel was taken under ultraviolet light, using an LAS 4000 Mini (FUJIFILM, Tokyo, Japan).

Cluster Analysis

From the photograph of the electrophoresed gel, species identification dots (*spiddos*), which indicate melting points (that is, the sequence-specific temperature at which double-stranded DNA unwinds and separates into single-stranded strands), were manually selected and adjusted using the *spiddos* of the reference internal standards. The pattern similarity scores (PaSS) were then calculated from obtained *spiddos* using micro TGGE analyzer software (Biyani and Nishigaki 2005; Kouduka et al. 2006, 2007; Nishigaki et al. 1984, 2000a; Naimuddin et al. 2000, 2002). PaSS values represent the degree of similarity between genomes. The value of PaSS ranges from 0 to 1.0. If the genome perfectly matches, then the PaSS value is 1.0. From the calculated PaSS values, all samples were cluster analyzed using the Ward method (Ward 1963).

RESULTS

The researchers obtained 17 *spiddos* from the samples and calculated the resulting PaSS values (Table 1). Representative images of the subsequent analyses are shown in Figure 1. These data indicate that the average PaSS value between cases was 0.9793 ± 0.0028 for blood samples, 0.9782 ± 0.0011 for buccal swabs, 0.979 ± 0.0002 for hair samples, and 0.9803 ± 0.0009 for nail samples. When investigating the PaSS value at the individual level, the average PaSS values against other sample types were 0.9881 ± 0.0004, 0.9882 ± 0.0011, 0.9821 ± 0.0029, 0.9833 ± 0.0049, and 0.9867 ± 0.0013 for cases 1 through 5, respectively.

Cluster analysis was performed by Ward's method, and all samples taken from a single individual were categorized as a single cluster (Fig. 2).

DISCUSSION

In a previous report, the GP method was reported to be inaccurate because of the need to select *spiddos* manually, which may account for an approximate one percent difference among the PaSS values. Nevertheless, because all samples taken from the same individual were categorized in a single cluster, the results indicate that it is possible to distinguish individuals by checking the cluster analysis of the GP result. Therefore, the researchers conclude that the GP method has potential for use in forensic identification based on blood, hair, nail, or saliva containing buccal cells found at crime scenes.

By comparing the samples taken on three occasions in one week per individual, the researchers also showed that each sample type was in a single cluster (data not shown). This indicates that the GP method may help estimate the sample type in addition to distinguishing between individuals. Of course, this result is limited by small sample, but given that the sample condition was relatively good, the researchers anticipate that further study with a larger sample size will confirm preliminary results and allow them to examine the effect of different sample conditions in future research.

Recently, next-generation sequencing has been used to detect SNPs or STR loci in the whole human genome or mitochondria genome for human identification (Pilli et al. 2016; Guo et al. 2016; Templeton et al. 2013; Li et al. 2017; Zhang et al. 2015). However, it requires increased

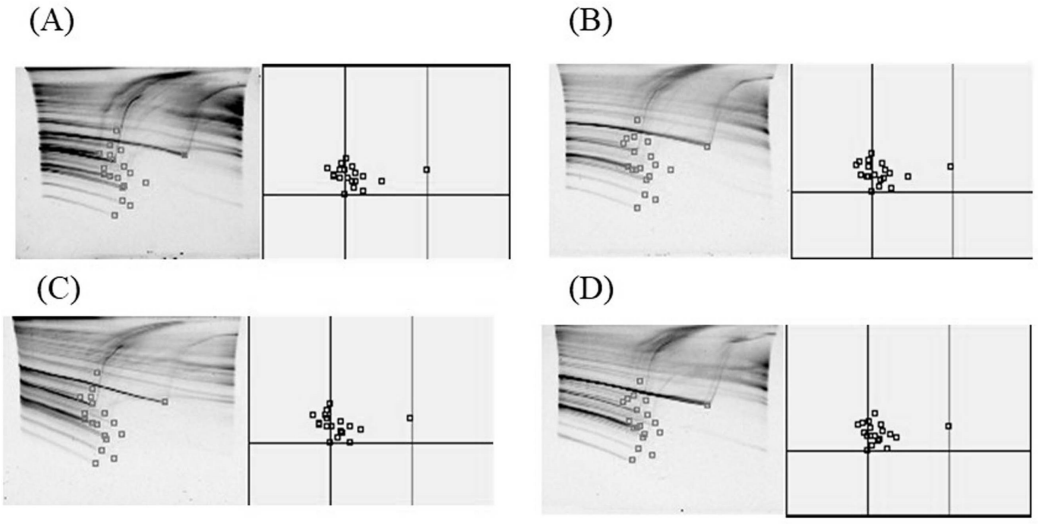

Fig. 1. Representative case of temperature gradient and "*spiddos*" patterns
The left images show the electrophoresis results, with open squares representing the *spiddos* (species identification dots). The right images show the standardized *spiddos* results based on the reference *spiddos*. (A) Blood, (B) nail, (C) buccal swab, and (D) hair

Table 1: Comparison of pattern similarity scores for five samples in five cases

	Bd(1)	*H(1)*	*N(1)*	*Bu(1)*	*Bd(2)*	*H(2)*	*N(2)*	*Bu(2)*	*Bd(3)*	*H(3)*	*N(3)*	*Bu(3)*	*Bd(4)*	*H(4)*	*N(4)*	*Bu(4)*	*Bd(5)*	*H(5)*	*N(5)*	*Bu(5)*
Bd(1)	1	0.9862	0.991	0.9891	0.9821	0.9737	0.9807	0.9817	0.9733	0.9801	0.9789	0.9799	0.9848	0.9788	0.9792	0.9755	0.9828	0.983	0.9807	0.9789
H(1)	0.9862	1	0.9863	0.9864	0.9838	0.9784	0.9834	0.9836	0.9733	0.981	0.9787	0.9759	0.9851	0.981	0.9821	0.9799	0.9831	0.9815	0.9828	0.9799
N(1)	0.991	0.9863	1	0.9876	0.9827	0.9749	0.9806	0.9803	0.9731	0.9794	0.9788	0.9777	0.9843	0.9789	0.98	0.9753	0.9823	0.9827	0.9805	0.9787
Bu(1)	0.9891	0.9864	0.9876	1	0.9798	0.9727	0.9779	0.9789	0.9743	0.9793	0.9754	0.9791	0.9822	0.9784	0.9792	0.9773	0.9819	0.9815	0.978	0.9794
Bd(2)	0.9821	0.9838	0.9827	0.9798	1	0.9863	0.9906	0.9892	0.9765	0.9822	0.9812	0.9806	0.9795	0.9791	0.9811	0.9762	0.9811	0.9796	0.9808	0.9803
H(2)	0.9737	0.9784	0.9749	0.9727	0.9863	1	0.9885	0.9863	0.978	0.9798	0.9814	0.9788	0.9736	0.9736	0.977	0.9707	0.9738	0.9726	0.9752	0.9749
N(2)	0.9807	0.9834	0.9806	0.9779	0.9906	0.9885	1	0.9907	0.9755	0.9807	0.9819	0.98	0.9785	0.9783	0.9817	0.9767	0.9807	0.9785	0.9814	0.9802
Bu(2)	0.9817	0.9836	0.9803	0.9789	0.9892	0.9863	0.9907	1	0.9757	0.9815	0.9808	0.9793	0.9797	0.9807	0.9825	0.9775	0.9796	0.9787	0.9784	0.9807
Bd(3)	0.9733	0.9733	0.9731	0.9743	0.9765	0.978	0.9755	0.9757	1	0.9862	0.9816	0.9825	0.9763	0.9738	0.9777	0.9732	0.9714	0.9733	0.9733	0.9713
H(3)	0.9801	0.981	0.9794	0.9793	0.9822	0.9798	0.9807	0.9815	0.9862	1	0.9866	0.9868	0.9806	0.9772	0.9794	0.9754	0.977	0.9777	0.9806	0.9759
N(3)	0.9789	0.9787	0.9788	0.9754	0.9812	0.9814	0.9819	0.9808	0.9816	0.9866	1	0.987	0.9782	0.9765	0.9787	0.9754	0.9773	0.978	0.9769	0.9797
Bu(3)	0.9799	0.9759	0.9777	0.9791	0.9806	0.9788	0.98	0.9793	0.9825	0.9868	0.987	1	0.9764	0.9761	0.9766	0.9736	0.9772	0.9789	0.9735	0.9779
Bd(4)	0.9848	0.9851	0.9843	0.9822	0.9795	0.9736	0.9785	0.9797	0.9763	0.9806	0.9782	0.9764	1	0.989	0.991	0.9871	0.9847	0.9844	0.9849	0.9778
H(4)	0.9788	0.981	0.9789	0.9784	0.9791	0.9736	0.9783	0.9807	0.9738	0.9772	0.9765	0.9761	0.989	1	0.9884	0.9907	0.9856	0.9839	0.9806	0.979
N(4)	0.9792	0.9821	0.98	0.9792	0.9811	0.977	0.9817	0.9825	0.9777	0.9794	0.9787	0.9766	0.991	0.9884	1	0.9887	0.9854	0.9845	0.9827	0.9772
Bu(4)	0.9755	0.9799	0.9753	0.9773	0.9762	0.9707	0.9767	0.9775	0.9732	0.9754	0.9754	0.9736	0.9871	0.9907	0.9887	1	0.9832	0.9829	0.98	0.978
Bd(5)	0.9828	0.9831	0.9823	0.9819	0.9811	0.9738	0.9807	0.9796	0.9714	0.977	0.9773	0.9772	0.9847	0.9856	0.9854	0.9832	1	0.992	0.9863	0.9876
H(5)	0.983	0.9815	0.9827	0.9815	0.9796	0.9726	0.9785	0.9787	0.9733	0.9777	0.978	0.9789	0.9844	0.9839	0.9845	0.9829	0.992	1	0.9841	0.9865
N(5)	0.9807	0.9828	0.9805	0.978	0.9808	0.9752	0.9814	0.9784	0.9733	0.9806	0.9769	0.9735	0.9849	0.9806	0.9827	0.98	0.9863	0.9841	1	0.9836
Bu(5)	0.9789	0.9799	0.9787	0.9794	0.9803	0.9749	0.9802	0.9807	0.9713	0.9759	0.9797	0.9779	0.9778	0.979	0.9772	0.978	0.9876	0.9865	0.9836	1

Blood = Bd; Hair = H; Nail = N; Buccal = Bu.

Note: The number in parentheses with the abbreviation is the case number from 1 through 5

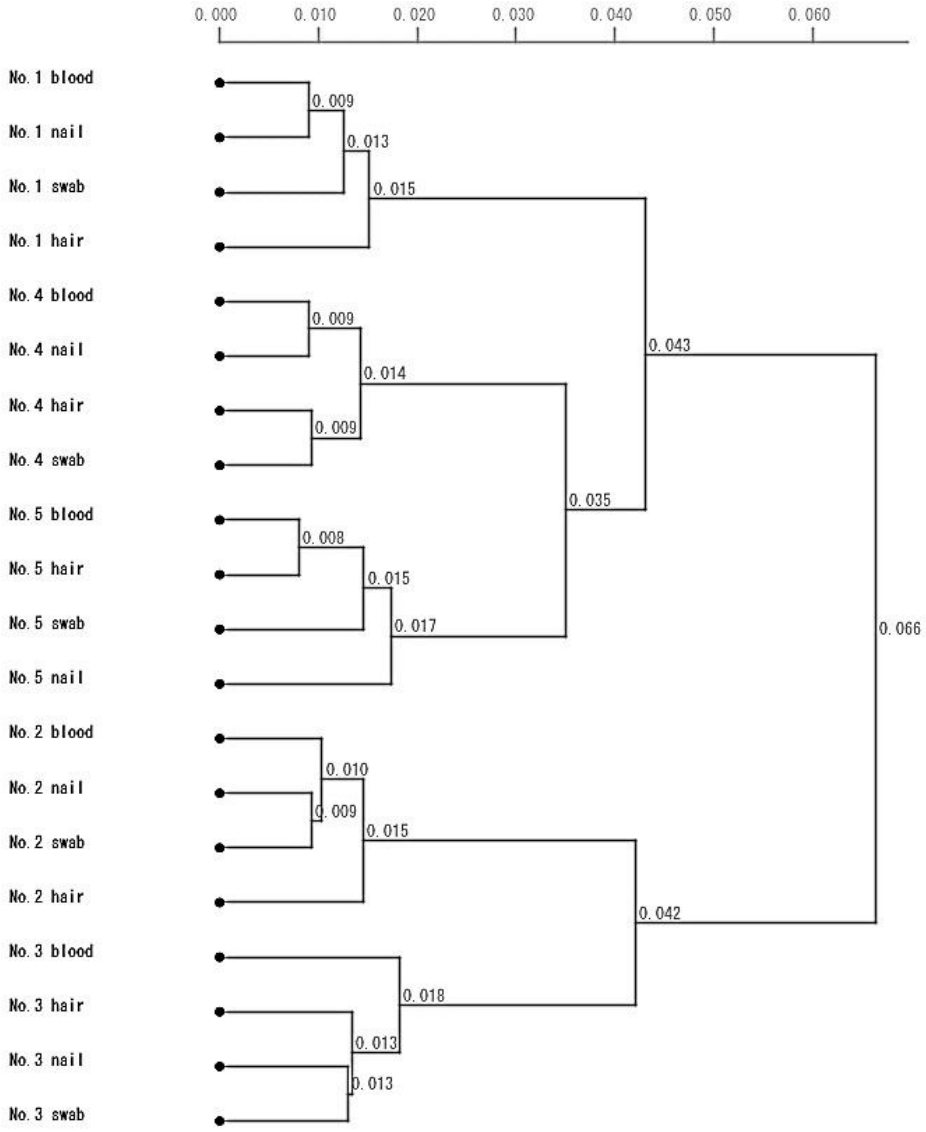

Fig. 2. Cluster analysis of biological material from five humans, using the Ward method
The number indicates the materials taken. Material samples from the same individuals are classified in the same clade. The phylogenetic tree was constructed by FreeLighter Version 0.02, Build 2005/11/10 (Daisuke Sato, All right reserved)

expense, skilled technique, and efforts. The GP method enables us to collect whole genome information easily by random PCR sampling. The required cost for the GP method is only one tenth of that of standard STR analysis, and it takes only a few hours to get the results. However, the researchers cannot recommend the GP method as anything but a screening tool. This is because it does not analyze the actual DNA sequence amplified by random PCR and because of the methodological issues in this report. Ultimately, there will be a need to test its non-inferiority against a generally accepted procedure, such as STR analysis.

CONCLUSION

The GP method can be used as a simple and economical screening tool for human identification.

RECOMMENDATIONS

The researchers also anticipate that automated spiddos selection methods will be developed, and that these will improve the accuracy of the GP method. At that point, this quick and cheap method could be proven suitable for widespread use in human identification.

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