FORENSIC SCIENCE JOURNAL SINCE 2002

Forensic Science Journal
2010;9(1):1-8
Available online at:fsjournal.cpu.edu.tw

STR genotyping of skin residues inside gloves

Li-Chin Tsai,[1]Ph.D.; James Chun-I Lee,[2,3]Ph.D.; Yu-Ming Lin,[1,4]M.S.; Pi-Yi Lai,[1]M.S.; Hsing-Mei Hsieh,[1,]*Ph.D.

[1]*Department of Forensic Science, Central Police University, 56 Shu-Jen Road, Kwei-San, Taoyuan 33334, Taiwan, R.O.C.*
[2]*Department of Forensic Medicine, College of Medicine, National Taiwan University, No.1 Jen-Ai Road Section 1, Taipei 10051, Taiwan, R.O.C.*
[3]*Institute of Forensic Medicine, Ministry of Justice, No. 166-1 Keelung Road Section 2, Taipei 10675, Taiwan, R.O.C.*
[4]*Forensic Section of Taichung City Police Bureau, 5F, No.588, Sec. 2, Wunsin Road, Situn District, Taichung City 40708, Taiwan, R.O.C.*

Received: April 23, 2010 / Accepted: June 09, 2010

Abstract

Evidences of gloves are frequently observed in the crime scene. In this study, skin residues attached on inside gloves were used as materials for evaluation of the strategy for STR typing of biological traces. The adhesive tapes and 2-propanol precipitation method were used for collecting samples and recovering DNA, respectively. DNA quantity and quality were evaluated by Real-time PCR and STR genotyping with AmpFISTR Cofiler PCR Amplification kit respectively. From the sensitivity test, it showed that while DNA from skin residues was not less than 0.43 ng, the full profile could be obtained with PCR amplification of 28 cycles as suggested. Increasing the PCR cycle number could enhance the STR identification while DNA was less than 0.5 ng. From the results, while DNA was not less than 0.5 ng, 28 cycles were suggested; 30 cycles for DNA ranged from 0.25 ng to less than 0.5 ng; and 32 cycles for DNA less than 0.25 ng. For simulated sample identification, the results showed that the full STR profile could be identified even for the gloves worn for 5 min and stored at room temperature for 6 months. The strategy provided in this study could be valuable on individualization of biological traces for forensic purposes.

Keywords: forensic science, biological traces, STR typing, real-time PCR

Introduction

For forensic scientists, it's more difficult than before to collect evidences in the crime scenes due to superior skills of suspects in extinguishing remained evidences. Sufficient biological evidences for DNA identification are also hard to be collected gradually. Therefore, it's urgent to develop the individualization system of low copy number DNA (LCN) [1] from human biological traces. DNA individualization of biological traces was studied in fingerprints [2, 3, 4, 5], in cases of hanging and ligature strangulation [6, 7], in evidence collecting from the inside of the victim's car [8], touched objects of stamps, pens and combs [9], and identification of trace DNA recovered from bedding [10]. The effect and efficiency of transfer were analyzed for the primary and secondary transfer [11, 12], collecting methods for many times transfer [13], and the tendency of individuals to transfer DNA to handled item [14]. Enhancement for LCN identification included a novel approach of miniSTR [15], improvement of PCR amplification [16], whole genome amplification methods [17, 18, 19], and pairing the ET cassette technology with the muCAE system [20]. However, artifacts of stutter, allele drop in, allele drop out and allele imbalance are usually observed for LCN

*Corresponding author: mei@mail.cpu.edu.tw

DNA identification [1, 21, 22, 23, 24, 25]. Therefore, interpreting low template DNA profiles were with more challenges [26]. Evidences of gloves are frequently observed in the crime scene, however, only few studies focus on the traces of epithelial cells remained on gloves [27, 28]. Therefore, skin residues attached on inside gloves were used as the materials in this study. PCR conditions were modified systematically to enhance the successful rate of STR typing. Simulated samples were also used to evaluate the strategy provided in this study for individualizing biological traces on gloves.

Materials and methods

Sample collection and DNA extraction

Samples were collected with informed consent. Oral swabs were used as references. Skin residues attached on inside gloves were collected by 1.5 cm x 1.5 cm of 3M adhesive tapes (3M, Bracknell, Berkshire, UK) for sensitivity and simulated tests. The skin residues were collected all over the insides of the gloves. Gloves used were of Vinyl materials and provided by Anderson Rubber Mate Co. (Chang Hwa, Taiwan). Other samples of skin residues from backs of the hands were collected for tests of PCR modifications. DNA from the collected samples was isolated by following the suggested protocols of DNA Extractor FM Kit (Wako, Osaka, Japan), by which DNA was precipitated with 2-propanol. In our previous study it showed that this method with the advantage for DNA extraction of biological traces (unpublished). Isolated DNA was quantified by Real-time PCR.

DNA quantification

Real-time PCR was performed by Quantifiler™ Human DNA quantification kit (Applied Biosystems, Foster City, CA, USA) and the machine of Applied Biosystems 7300 Real-time PCR. The r^2 value of the quantification curves was over 0.99. DNA concentration for each sample was adopted as the average of triplicate results.

STR genotyping

STR typing was performed by AmpFlSTR® COfiler™ kit (Applied Biosystems) and Applied Biosystems 310 DNA Analyzer. Totally there were 6 STR loci (D3S1358, TH01, TPOX, D16S539, D7S820 and CSF1PO) and the amelogenin gene typed. As the manufacture's protocol, template DNA of 1-2.5 ng was suggested, and the PCR condition was 95 ºC for 11 min, 28 cycles of 94 ºC for 1 min, 59 ºC for 1 min and 72 ºC for 1 min, and further extension at 64 ºC for 45 min. For PCR modifications, template DNA ranged from 0.016 to 1 ng and the cycle number was 28, 30, 32 or 34.

Results and discussion

Sensitivity test

For the AmpFISTR® Cofiler PCR Amplification kit, the recommended range of input DNA is 1.0-2.5 ng and it has been used successfully in typing the sample containing less than 1 ng of DNA (AmpFISTR Cofiler PCR Amplification Kit User Bulletin). The sensitivity of skin residues for STR typing with this kit was determined in this study. Samples of the skin residues on gloves worn for 10 min by one subject were collected. The recovered DNA was quantified by Real-time PCR quantification method. The isolated DNA was 13.4±4.4 ng from three independent experiments and enough for the further STR typing. Variation of the total DNA amount was usually dependent on the individual nature, and time length and action during wearing the gloves. DNA quality was evaluated by STR genotyping with AmpFISTR® Cofiler PCR Amplification kit following the suggestion of the manufacture's protocol. Different DNA input was used to evaluate the sensitivity in this study. The amount of DNA input for STR typing was based on its concentration for taking convenience. Totally there were 8 DNA inputs evaluated. The threshold of 50 RFU was used for peak determination. The results were showed in Table 1. Number of total alleles was 14 for the loci of six STR and amelogenin. The result indicated that while DNA from skin residues was not less than 0.43 ng, the full profile could be observed with the suggested protocol of PCR amplification. While DNA was less, allele dropout was more serious. Allele drop out was frequently observed for typing LCN DNA [1, 23, 24]. Allele drop in was not observed in this case. Figure 1 showed the electropherogram of one example. Full profile was observed except that with 0.17 ng DNA template (Figure 1 (B)), in which there were 4 alleles dropped out.

Table 1. Sensitivity test of STR typing for skin residues.

DNA input (ng)	Identified allele no.	Drop out allele no.
0.05	5	9
0.17	10	4
0.28	12	2
0.36	13	1
0.43	14	0
0.64	14	0
0.78	14	0
1.00	14	0

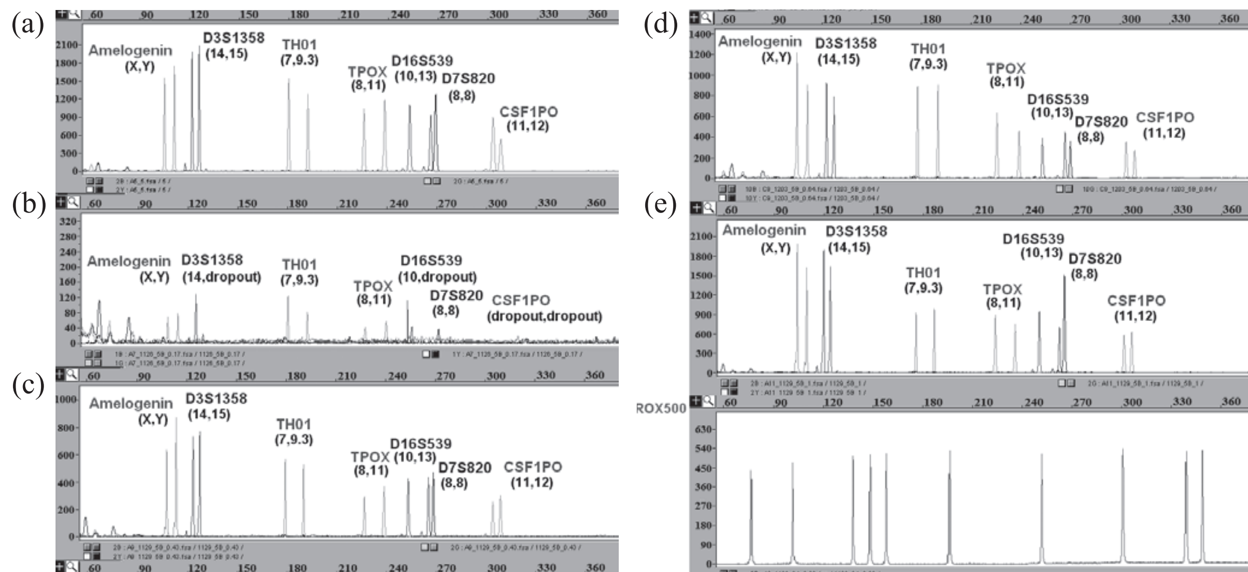

Fig.1 The electropherogram of one example. DNA from oral swab for reference (A) and skin residues (B to E) was typed with AmpFlSTR® COfiler™ kit. DNA template was 0.17 ng (B), 0.43 ng (C), 0.64 ng (D) and 1 ng (A and E) respectively.

PCR modifications for STR typing

To enhance the successful rate of STR typing with LCN DNA, modifications of PCR conditions were performed in this study. DNA with a serial dilution from 1, 0.5, 0.25, 0.125, 0.063, 0.032 to 0.016 ng were extracted from the skin residues of backs of the hands for three subjects and typed by AmpFlSTR® COfiler™ kit for amplification of 28, 30, 32 or 34 cycles. The result was showed in Table 2. PCR amplification for 28 cycles as the manufacture's suggest, alleles dropped

Table 2. Variations of the STR genotyping with a serial dilution of DNA and amplification of different cycles.

DNA Input (ng)	Cycle no. of PCR[a]											
	28			30			32			34		
	Identified allele no.	Allele drop out no.	Allele drop in no.	Identified allele no.	Allele drop out no.	Allele drop in no.	Identified allele no.	Allele drop out no.	Allele drop in no.	Identified allele no.	Allele drop out no.	Allele drop in no.
0.016	4±4.58	10±4.58	0	7.3±4.62	6.7±4.62	0	7.3±3.51	6.7±3.51	0	8.7±5.51	5.3±5.51	0
0.032	6.3±4.04	7.7±4.04	0	10.7±3.21	3.3±3.21	0	8.7±4.73	5.3±4.73	0	8±5.57	6±5.57	0.7±1.15
0.063	10.3±3.21	3.7±3.2	0	13.3±1.15	0.7±1.2	0	12±2.65	2±2.6	0	11±4.36	3±4.4	2±1.7
0.125	12±2	2±2	0	10±6.93	4±6.9	0	14.0±0	0	0	12.7±2.31	1.3±2.3	3.3±1.2
0.25	14.0±0	0	0	14.0±0	0	0	14.0±0	0	3±1.73	13.8±0.5	0.3±0.5	4.3±1.53
0.5	14.0±0	0	0	14.0±0	0	0.3±0.58	14.0±0	0	3.7±0.58	14.0±0	0	5±0
1	14.0±0	0	0	14.0±0	0	1±1.7	14.0±0	0	3.3±2.1	14.0±0	0	5.7±0.6

[a]The numerical data represented the average for three subjects.

out observed obviously while DNA was not more than 0.125 ng. Increasing the cycle number, the number of successfully identified alleles increased. However, allele dropped in observed obviously while the cycle number was 32 for not less than 0.25 ng DNA. The cycle number increased to 34, allele dropped in was more serious. Allele imbalance was also evaluated in this study. Comparing with the STR types of the reference, allele imbalance was indicated while the ratio of lower quantity peak and higher quantity peak was less than 70 % of heterozygote loci [29]. The results showed that no. of the allele imbalance loci was unrelated with the DNA input and amplification cycles. However, it was observed that the level of allele imbalance was more serious while DNA was less. Figure 2 showed the electropherogram of one example of 0.125 ng DNA amplification for 28, 30, 32 and 34 cycles. Allele dropped out was observed on TPOX locus for 28 cycle amplification in Figure 2 (A). Allele imbalance was serious on D3S1358 locus for 30 cycle amplification in Figure 2 (B). Pull up signals were observed on the loci of Amelogenin, D3S1358, TH01, TPOX, D16S539 and CSF1PO for 34 cycle amplification in Figure 2 (D).

Considering the effects of artifacts, such as allele drop out, allele drop in, allele imbalance and pull up, it was suggested that while DNA was not less than 0.5 ng, 28 cycles were suggested; 30 cycles for DNA ranged from 0.25 ng to less than 0.5 ng; and 32 cycles for DNA less than 0.25 ng. Amplification of 34 cycles was not suggested due to the serious artifacts, such as allele dropped in and pull up.

Simulated tests

Simulated samples were used to evaluate the strategy provided in this study. The successful rate and the effects of different storage period for the simulated

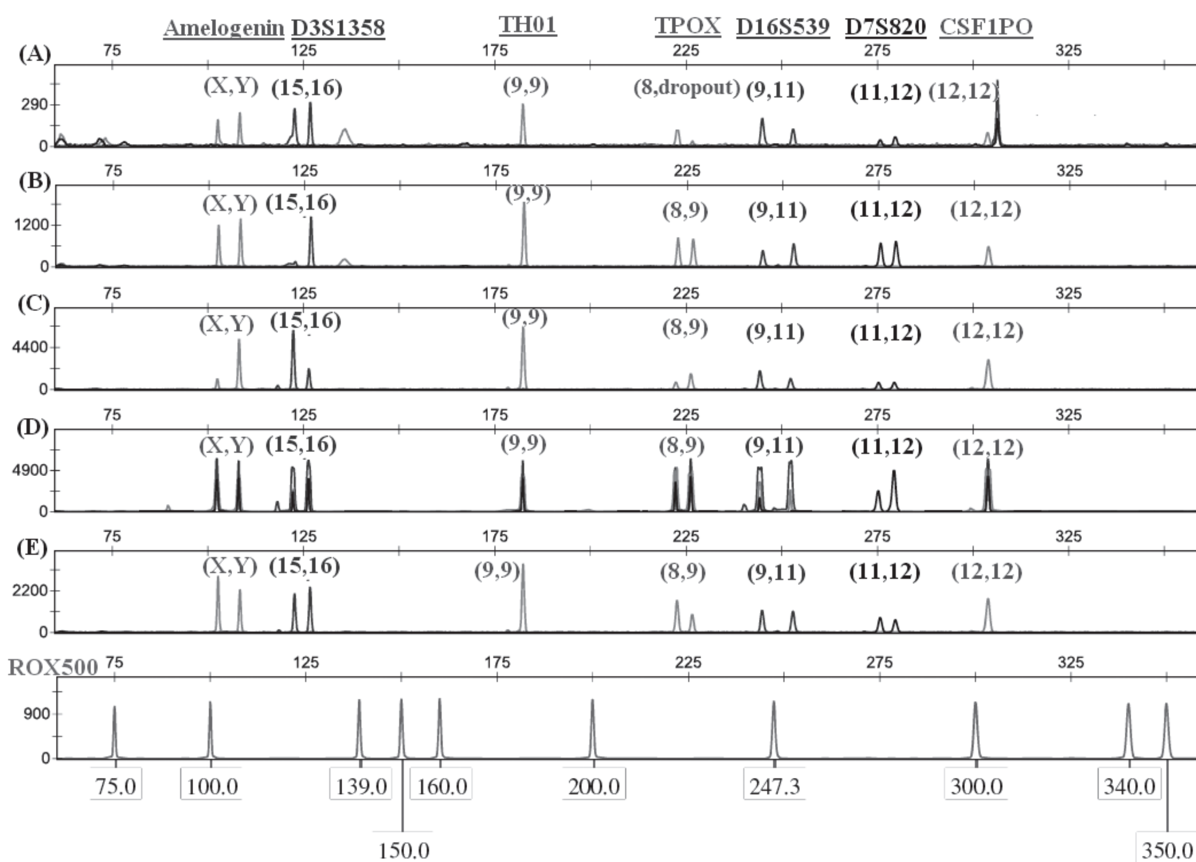

Fig.2 The electropherogram of one example of 0.125 ng DNA amplification for 28 (A), 30 (B), 32 (C) and 34 (D) cycles. (E) 1ng DNA from reference sample was amplified for 28 cycles.

Table 3. STR genotyping of 10 subjects for simulated test.

Subject[a]	DNA input (ng)	Cycle no.	Identified allele no.[b]	Drop in allele no.
1A	1.15	28	14	
1B	0.73	28	12	
2A	1.32	28	14	
2B	1.09	28	10	
3A	1.23	28	14	
3B	0.86	28	14	
4A	0.62	28	14	
4B	0.75	28	14	
5A	1.34	28	12	2
5B	0.62	28	9	
6A	0.91	28	14	
6B	1.24	28	14	
7A	1.17	28	14	
7B	0.62	28	10	
8A	0.99	28	14	
8B	1.16	28	14	
9A	1.09	28	14	
9B	1.02	28	14	
10A	0.17	32	1	
10B	0.23	32	1	

[a]A and B represent the left or right glove randomly, and are used for duplicate experiments.
[b]Total allele number of each sample was calculated as 14.

samples were measured. Skin residues from pair of the gloves were extracted separately as the duplicate samples. The gloves worn for 10 min by each of the 10 subjects and stored at room temperature for 4 months were used as the simulated samples. Cycle number of PCR amplification for STR typing was determined following the above suggestions. The result was showed in Table 3. DNA input for STR typing was based on its concentration for taking convenience or the total amount of isolated DNA. Input DNA was used about 1 ng as far as possible. However, DNA from some samples was under the limit and, therefore, the total DNA was condensed for analysis. The results showed that the successful rate was 80 % while full profiles of all the alleles were unambiguously identified for either one of the pair of gloves. Subject 10 could be a poor shedder. While DNA was more than 1 ng, the full profile could be observed except for 2B and 5A in Table 3. Long time storage of samples could cause DNA to be degraded.

Other simulated samples were the gloves worn for 30 sec, 1 min, 5 min, 10 min and 30 min from one subject and stored at room temperature for 6 and 18 months. The

results showed that the full STR profile of skin residues on gloves worn for at least 5 min and stored for 6 months could be identified successfully. Not more than half alleles were observed while the gloves were stored for 18 months. The identified alleles were 2, 5 and 6 for the gloves worn for 5 min, 10 min and 30 min respectively. It was dependent on time length of gloves worn. Only few alleles were identified in these cases due to the highly degraded nature of DNA when stored for a long time at room temperature. However, the results for the simulated tests showed that the skin residue attached on inside gloves were worthful trace evidences and the strategy provided in this study could be valuable on forensic applications.

Conclusions

In this study the strategy was provided for STR typing of skin residues inside gloves. The optimal conditions were reported and it will be helpful for unambiguously interpreting the STR profile of biological traces.

Acknowledgements

This study was supported by NSC96-2320-B-015-002-MY2 and 98-2320-B-015-001-MY3 from National Science Council and Department of Medical Research in NTUH, Taiwan, ROC.

References

1. Gill P, Whitaker JP, Flaxman C, Brown N, Buckleton J. An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. Forensic Sci. Int. 2000; 112:17-40.
2. Van Oorschot RAH, Jones MJ. DNA fingerprints from fingerprints. Nature 1997; 387:767.
3. Balogh MK, Burger J, Bender K, Schneider PM, Alt KW. STR genotyping and mtDNA sequencing of latent fingerprint on paper. Forensic Sci. Int. 2003; 137:188-195.
4. Leemans P, Vandeput A, Vanderheyden N, Cassiman J, Decorte R. Evaluation of methodology for the isolation and analysis of LCN-DNA before and after dactyloscopic enhancement of fingerprints. Int. Congress Ser. 2006; 1288:583-585.
5. Sewell J, Quinones I, Ames C, Multaney B, Curtis S, Seeboruth H, Moore S, Daniel B. Recovery of DNA and fingerprints from touched documents. Forensic Sci. Int. Genet. 2008; 2:281-285.
6. Wiegand P, Kleiber M. DNA typing of epithelial cells after strangulation. Int. J. Legal Med. 1997; 110:181-183.
7. Bohnert M, Faller-Marquardt M, Lutz S, Amberg R, Weisser H, Pollak S. Transfer of biological traces in cases of hanging and ligature strangulation. Forensic Sci. Int. 2001; 116:107-115.
8. Pizzamiglio M, Mameli A, My D, Garofano L. Forensic identification of a murderer by LCN DNA collected from the inside of the victim's car. Int. Congress Ser. 2004; 1261:437-439.
9. Barbaro A, Cormaci P, Barbaro A. LCN DNA typing from touched objects. Int. Congress Ser. 2006; 1288:553-555.
10. Petricevic SF, Bright J, Cockerton SL. DNA profiling of trace DNA recovered from bedding. Forensic Sci. Int. 2006; 159:21-26.
11. Lowe A, Murray C, Whitaker JP, Tully G, Gill P. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. Forensic Sci. Int. 2002; 129:25-34.
12. Lowe A, Murray C, Richardson P, Wivell R, Gill P, Tully G, Whitaker JP. Use of low copy number DNA in forensic inference. Int. Congress Ser. 2003; 1239:799-801.
13. Van Oorschot RAH, Phelan DG, Furlong S, Scarfo GM, Holding NL, Cummins MJ. Are you collecting all the available DNA from touched objects? Int. Congress Ser. 2003; 1239:803-807.
14. Phipps M, Petricevic S. The tendency of individuals to transfer DNA to handled item. Forensic Sci. Int. 2007; 168:162-168.
15. Romano C, Di Luise E, Di Martino D, Spitaleri S, Saravo L. A novel approach for genotyping of LCN-DNA recovered from highly degraded samples. Int. Congress Ser. 2006; 1288:577-579.
16. Anjos MJ, Andrade L, Carvalho M, Lopes V, Serra A, Oliveira C, Batista L, Balsa F, Brito P, Corte-Real F, Vide MC. Low copy number: Interpretation of evidence results. Int. Congress Ser. 2006; 1288:616-618.
17. Ballantyne KN, Van Oorschot RAH, Mitchell RJ. Comparison of two whole genome amplification methods for STR genotyping of LCN and degraded DNA samples. Forensic Sci. Int. 2007; 166:35-41.
18. Sorensen KM, Jespersgaard C, Vuust J, Hougaard D, Norgaard-Pedersen B, Andersen PS. Whole genome

amplification on DNA from filter paper blood spot samples: an evaluation of selected systems. Genet. Test. 2007; 11:65-71

19. Barber AL, Foran MS, Foran DR. The utility of whole genome amplification for typing compromised forensic samples. J. Forensic Sci. 2006; 51:1344-1349.
20. Yeung SH, Seo TS, Crouse CA, Greenspoon SA, Chiesl TN, Ban JD, Mathies RA. Fluorescence energy transfer-labeled primers for high-performance forensic DNA profiling. Electrophoresis 2008; 29:2251-2259.
21. Gill P. Application of low copy number DNA profiling. Croatian Med. J. 2001; 42:229-232.
22. Whitaker JP, Cotton EA, Gill P. A comparison of the characteristics of profiles produced with the AMPFlSTRR SGM Plusk multiplex system for both standard and low copy number (LCN) STR DNA analysis. Forensic Sci. Int. 2001; 123:215-223.
23. Kloosterman AD, Kersbergen P. Efficacy and limits of genotyping low copy number DNA samples by multiplex PCR of STR loci. Int. Congress Ser. 2003; 1239:795-798.
24. Caragine T, Mikulasovich R, Tamariz J, Bajda E, Sebestyen J, Baum H, Prinz M. Validation of testing and interpretation protocols for low template DNA samples using AmpFlSTR Identifiler. Croatian Med. J. 2009; 50:250-267.
25. Budowle B, Eisenberg AJ, Daal AV. Validity of low copy number typing and applications to forensic science. Croatian Med. J. 2009; 50:207-217.
26. Balding DJ, Buckleton J. Interpreting low template DNA profiles. Forensic Sci. Int. Genet. 2009; 4:1-10.
27. Förster R, Eberspächer B. Evidence of DNA from epithelial cells of the hand of the suspect found on the tool of the crime. Arch. Kriminol. 1999; 203:45-53.
28. Pizzamiglio M, Marino A, Maugeri G, Stabile M, Garofano L. The importance of a well defined analytical strategy to solve complex murder cases. Int. Congress Ser. 2006; 1288:642-644.
29. Gill P, Sparkes R, Kimpton C. Development of guidelines to designate alleles using an STR multiplex system. Forensic Sci. Int. 1997; 89:185-197.

警察大學 CENTRAL POLICE UNIVERSITY