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TECHNICAL NOTE

Su Jeong Park,[1] Ph.D.; Jong Yeol Kim,[2] M.S.; Young Geun Yang,[2] Ph.D.; and Seung Hwan Lee,[1] Ph.D.

Direct STR Amplification from Whole Blood and Blood- or Saliva-Spotted FTA® without DNA Purification*

ABSTRACT: The DNA purification step has been thought to be essential for typing of STR DNA. However, this process is time-consuming, and there is a risk of unexpected cross-contamination during purification. We report a new method for direct short tandem repeat (STR) amplification using a newly developed direct PCR buffer, AnyDirect™, which can amplify STR loci from whole blood and blood- or saliva-spotted FTA® cards without DNA purification. The autosomal and Y chromosomal STR loci were analyzed for whole blood and blood or saliva spots of random individuals, followed by comparison of the results with those of corresponding purified DNA. The results from whole blood and blood spots showed perfect concordance with those from purified DNA without allele or locus drop-out. However, in the case of saliva spots, no amplification or locus drop-out was observed in some of the samples, which offers a topic for further study. Additionally, some commercial hot-start DNA polymerases other than AmpliTaq Gold® DNA polymerase were also found to be compatible with this buffer system. Therefore, this direct PCR buffer was demonstrated to be useful for fast forensic DNA analysis or criminal DNA databases for which there is no need to store DNA samples.

KEYWORDS: forensic science, DNA typing, direct polymerase chain reaction, short tandem repeat, blood, saliva, DNA database

Various protocols have been devised for the extraction of genomic DNA from human specimens; these methods are based on boiling, hydrolysis with proteinase K, and treatment with detergents or alkali (1–4). The recent development of many commercialized DNA extraction kits has made DNA purification far easier and faster than the standard procedures listed above. However, these new methods still require considerable time and expense, especially for a massive project such as a criminal DNA database that necessitates high-throughput and economical efficiency. The development of a reliable direct PCR system could be very helpful to those countries that do not store the DNA of offenders in case files. A direct PCR amplification system offers the advantages of time- and cost-efficiency, convenience, prevention of the loss of trace samples during DNA extraction, and avoidance of infection in sample handlers. Most biological specimens contain various PCR inhibitors such as polysaccharides, proteins, lipids, and salts that prevent direct PCR amplification (5,6). We have developed a direct PCR buffer system that can overcome typical PCR inhibition from blood, saliva, and plant materials (7,8). Thus, we investigated direct short tandem repeat (STR) amplification using a direct buffer system and a commercialized human identification kit (9) such as AmpFℓSTR Identifiler® (Applied Biosystems, Foster City, CA), AmpFℓSTR Yfiler™ (Applied Biosystems), and Powerplex®Y (Promega, Madison, WI). Future studies will improve on this method, making it appropriate for use on forensic samples found at violent crime scenes.

[1]DNA Analysis Lab, Forensic Science Division, Supreme Prosecutors' Office, Seoul 137-730, Korea.
[2]BioQuest, Inc., Seoul 136-865, Korea.

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Materials and Methods

Blood and Saliva Specimens

Peripheral blood and saliva samples were collected from 50 volunteers after obtaining informed consent. Peripheral bloods were collected in an EDTA-Vacutainer®. For direct PCR from blood, specimens were diluted to 1:120 (v/v) with distilled water just prior to PCR. Blood spots were prepared by spotting 100 μL blood on a 2.5 cm diameter FTA® card (Whatman, Florham Park, NJ). For saliva, buccal swabs were collected using a sterile, foam-tipped applicator (Whatman) and smeared onto the FTA® indicating card (Whatman). The cards were then dried and stored at room temperature. For the concordance test, genomic DNAs were purified from blood or saliva with a NucleoSpin® tissue kit (Macherey-Nagel, Easton, PA).

STR Amplifications and Fragment Analysis

A 2-mm diameter punch of a blood- or saliva-spotted FTA® card was placed into a 1.5-mL test tube and immersed in 40-μL Direct-N-Elute™ elution buffer (BioQuest, Seoul, Korea) for 15 min at room temperature with intermittent gentle vortexing. One-microliter aliquot was used as a template for a 10-μL PCR reaction. For direct PCR from blood, a 1-μL aliquot of diluted whole blood (1:120, v/v) was used. Each PCR reaction was performed with AmpFℓSTR Identifiler®, AmpFℓSTR Yfiler™, or Powerplex®Y as described in the manual provided by the

manufacturer, except that the PCR buffer in the kit was substituted with direct PCR buffer (AnyDirect™ PCR buffer, BioQuest). AnyDirect™ PCR buffer is mainly composed of zwitterionic buffer and/or nonreducing carbohydrates to overcome the PCR inhibitory effects as well as dNTPs (10). HotStarTaq™ DNA polymerase (Qiagen, Hilden, Germany) was sometimes used instead of AmpliTaq Gold® DNA polymerase (Applied Biosystems) in the kits. Simple schematic procedures are illustrated in Fig. 1.

After amplification, 1.5 μL of PCR products were loaded onto an ABI 3100-Avant genetic analyzer (Applied Biosystems), and the collected data were analyzed using GeneScan V3.7 and Genotyper V3.7 NT software (Applied Biosystems).

Results and Discussion

Direct Amplification From Blood

The initial testing of direct PCR amplification was focused on whether direct PCR buffer could be used to directly amplify whole blood without any pretreatment. To determine the proper dilution fold for amplification without DNA quantitation, blood was serially diluted from 1:20 (v/v) to 1:120 (v/v). Among the dilutions, the 1:120 (v/v) dilution batch showed optimal amplification results, while others showed either too many peaks or low signals. Considering that 1 μL of blood has 30–40 ng of DNA on average, the amount of DNA in 1 μL of 1:120 (v/v) diluted blood is estimated to 0.25–0.33 ng, which is coincident with the optimal template amount for amplification. Control 9947A DNA and diluted blood (1:120) was amplified with either conventional buffer or AnyDirect™ PCR buffer with variations in DNA polymerases. As shown in Fig. 2, the conventional PCR buffer could not amplify the blood samples (S5 and S6), while AnyDirect™ PCR buffer successfully amplified blood with both kinds of DNA polymerases (S7 and S8). AnyDirect™ PCR buffer was also able to amplify purified control DNA, showing an even higher RFU value than conventional PCR buffer (S1 and S3, S2 and S4). The quality of amplification from diluted blood was as good as that from control DNA in terms of normal peak shape and height, and the lack of baseline noise or stutter bands.

Concordance Test of Blood or Saliva Spots on FTA®

As in diluted blood, direct PCR from blood and saliva spots was also tested (Fig. 3). All amplification results from 50 blood spots fully matched those from corresponding purified DNA, showing no locus or allele drop-out in any of the STR loci of AmpFℓSTR Identifiler®. However, in 19 of 50 saliva spots, no or poor amplification occurred under the normal elution conditions described in Materials and Methods. When these saliva spots extracts were further diluted by twofold with Direct-N-Elute™ elution buffer, amplification could be improved to show no locus drop-out, with a peak height of more than 100 RFU in four cases. However, 15 saliva spots remained poorly amplified with some locus drop-out. This suggests that amplification failure of saliva spots results from the presence of PCR inhibitors in saliva, rather than from the concentration of DNA. Actually, the amount of DNA or chemical composition of saliva could not be made homogeneous among individuals. Therefore, this remains a topic of further study for the AnyDirect™ PCR buffer system.

Peak Heights and Imbalances

Amplification results from AmpFℓSTR Identifiler® were analyzed for their peak heights and imbalances. Peak heights were calculated as follows to represent average height of a peak in heterozygotes: $(P_1 + P_2)/2$ in heterozygotes and $P_1/2$ in homozygotes (P_1 and P_2 represent the peak height of each allele). Table 1 shows the summary of peak heights (minimum, mean, median) for 50 blood samples, 50 blood spots, and 35 saliva spots, respectively, which showed total concordance with their corresponding purified DNA. The overall peak heights were low in saliva spots relative to blood or blood spots, particularly in large allele loci. Peak imbalances are also summarized in Table 2. Peak height ratios were calculated for heterozygous samples as follows: P_L/P_H (P_L: lower peak height;

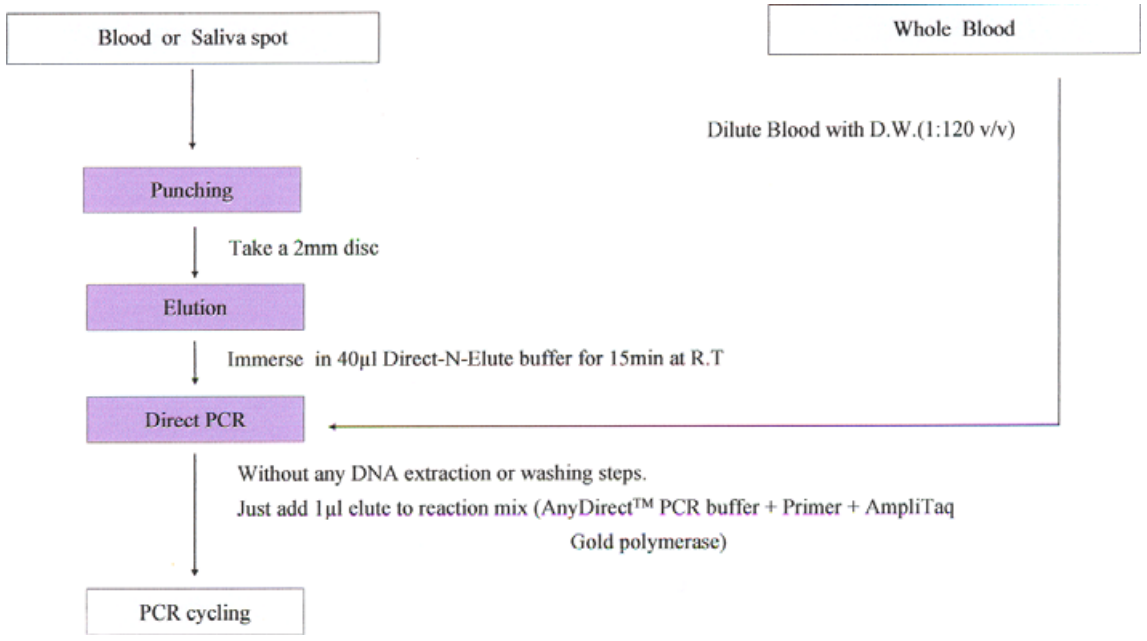

FIG. 1—*Flow chart of direct PCR.*

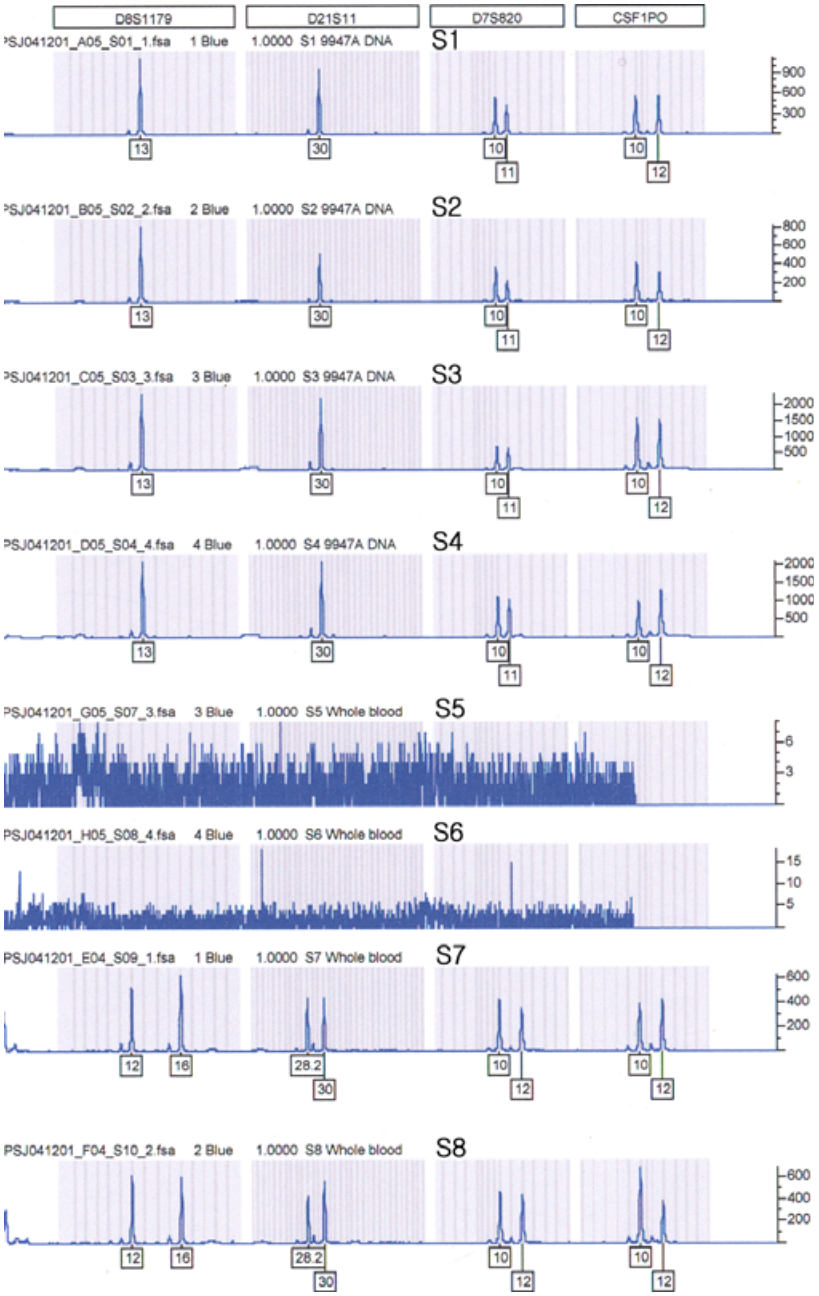

FIG. 2—*Electropherograms of direct PCR amplification from whole blood with AnyDirect buffer and AmpFℓSTR buffer. S1: DNA(9947A) + AmpFℓSTR buffer + AmpliTaq Gold; S2: DNA (9947A) + AmpFℓSTR buffer + HotStarTaq; S3: DNA(9947A) + AnyDirect PCR buffer + AmpliTaq Gold; S4: DNA(9947A) + AnyDirect PCR buffer + HotStarTaq; S5: Whole blood (1/120x) + AmpFℓSTR buffer + AmpliTaq Gold; S6: Whole blood (1/120x) + AmpFℓSTR buffer + HotStarTaq; S7: Whole blood (1/120x) + AnyDirect PCR buffer + AmpliTaq Gold; S8: Whole blood (1/120x) + AnyDirect PCR buffer + HotStarTaq.*

P_H: higher peak height). Overall peak height ratios were relatively less than those shown in the AmpFℓSTR Identifiler® User's Manual. Particularly in terms of minimal values, some blood and saliva spots had peak height ratios below 0.4, which are not observed in the data noted in the AmpFℓSTR Identifiler® User's Manual.

Amplification From Old Blood Spots on FTA®

FTA® cards are widely used for stable storage of blood or saliva over an extended period of time. Twenty 1–2-year-old blood spots on FTA® were tested for direct PCR amplification with an AmpFℓSTR Identifiler® kit. All of the tested samples were amplified at all loci. Figure 4 shows one example of the results of direct PCR amplification from a 2-year-old blood spot compared with fresh blood and saliva spots. The results showed that AnyDirect™ PCR buffer amplified the old blood spot at a quality almost equal to that of the fresh spots. This suggests that the direct PCR system can be applied to old samples that are spotted on FTA®, and are then dried and stored at room temperature.

After Elution Time Interval Test of Direct PCR

Five blood and five saliva spots were amplified at different time intervals (0, 1 and 3 days, 1 and 2 weeks, and 1 month after dilution with Direct-N-Elute™ elution buffer and storage in refrigerator), and the results were compared with each other. Figure 5

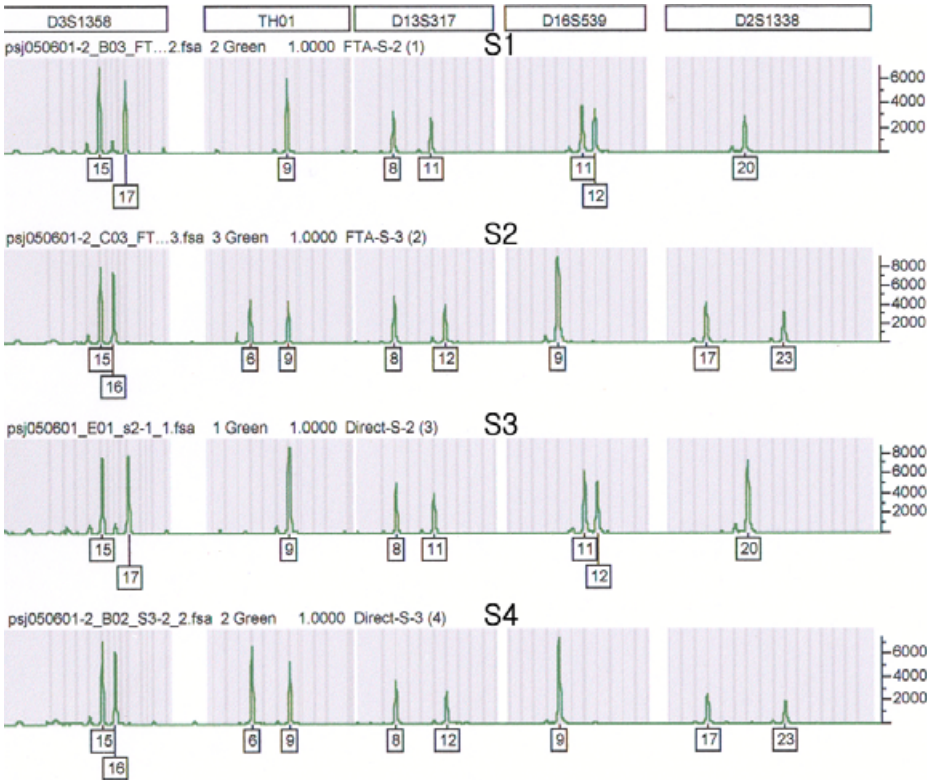

FIG. 3—*Direct PCR from blood and saliva spots was compared with that from purified DNA. S1–2: Amplification from purified DNA; S3: amplification from blood spot; S4: amplification from saliva spot.*

TABLE 1—*Comparison of peak heights.**

	Blood (n = 50)				Blood spot (n = 50)				Saliva spot (n = 35)			
	Min	Max	Mean	Med†	Min	Max	Mean	Med	Min	Max	Mean	Med
D8S1179	387	4016	1447	1379	136	3495	1203	969	156	4906	842	517
D21S11	233	2399	860	658	70	1465	542	507	120	2452	426	261
D7S820	191	2578	965	784	58	1787	691	634	101	2492	522	372
CSF1PO	174	4654	1571	1442	225	2541	1055	919	165	2422	742	587
D3S1358	298	3357	1111	939	166	3284	1160	874	337	7665	1578	1073
TH01	68	3650	1045	992	134	3189	1154	948	292	6720	1333	881
D13S317	650	7088	2175	1876	124	2081	863	797	120	5274	981	571
D16S539	211	3667	1135	874	106	2498	904	695	219	5703	873	545
D2S1338	51	2973	922	836	129	2137	874	691	155	3646	660	490
D19S433	172	2912	1059	913	206	3159	1155	989	310	5302	1034	673
VWA	271	4866	1570	1347	356	4219	1831	1720	328	8184	1315	815
TPOX	52	3414	1092	950	229	4208	1558	1158	197	8310	1273	843
D18S51	437	5206	1732	1442	196	2593	1066	1044	86	3919	716	496
D5S818	241	2720	986	922	80	2243	747	575	175	5265	852	534
FGA	148	2374	815	682	52	1450	538	469	94	4115	652	408
Amel	324	5204	1712	1604	544	6137	2286	2206	414	7388	1808	1290

*Peak heights were collected from those samples that showed complete concordance with their corresponding DNA without any locus drop-out.
†Median value.

shows the results for blood and saliva spots at 0 days and after 1 month. No significant differences were shown in peak heights between two electropherograms. All profiles of six time-interval spots matched completely.

Effect of Primer Concentration on Direct PCR

The relative amplification quality from previously FTA®-spotted blood was compared according to reduced primer concentration (Table 3). Among nine tested spots, no dramatic reduction in peak height was observed between a half volume of primer and the recommendations of the manufacturers in all three amplification kits. However, when reduced to one-third of the recommended volume, peak height was significantly reduced in all tested kits, and even allele drop-outs were observed in the case of Powerplex®Y.

Conclusions

In this study, a system for direct PCR amplification (AnyDirect™ PCR buffer) from blood and blood/saliva spots was developed to replace conventional DNA amplification, which requires purification of DNA. In respect to concordance with

TABLE 2—*Comparison of peak height ratios.**

	Blood			Blood spot			Saliva spot			Identifiler® manual†		
	Min	Mean	Med‡	Min	Mean	Med	Min	Mean	Med	Min	Mean	Med
D8S1179	0.50	0.83	0.85	0.44	0.78	0.78	0.42	0.81	0.84	0.58	0.90	0.93
D21S11	0.54	0.82	0.81	0.49	0.80	0.82	0.46	0.80	0.83	0.66	0.88	0.89
D7S820	0.49	0.83	0.86	0.47	0.78	0.83	0.48	0.78	0.79	0.66	0.89	0.90
CSF1PO	0.49	0.85	0.90	0.39	0.80	0.81	0.37	0.71	0.71	0.64	0.86	0.88
D3S1358	0.61	0.86	0.89	0.45	0.82	0.82	0.50	0.83	0.85	0.64	0.88	0.90
TH01	0.41	0.74	0.71	0.38	0.73	0.75	0.47	0.82	0.87	0.49	0.86	0.88
D13S317	0.61	0.85	0.85	0.51	0.81	0.83	0.34	0.80	0.84	0.63	0.87	0.87
D16S539	0.59	0.82	0.84	0.41	0.78	0.76	0.30	0.79	0.84	0.62	0.88	0.91
D2S1338	0.50	0.82	0.85	0.42	0.75	0.79	0.45	0.71	0.74	0.43	0.84	0.86
D19S433	0.42	0.72	0.69	0.42	0.77	0.78	0.24	0.72	0.79	0.49	0.88	0.92
VWA	0.64	0.82	0.81	0.48	0.76	0.79	0.56	0.77	0.78	0.63	0.86	0.88
TPOX	0.41	0.80	0.84	0.52	0.79	0.81	0.49	0.79	0.78	0.56	0.87	0.92
D18S51	0.60	0.83	0.84	0.37	0.75	0.75	0.50	0.76	0.79	0.56	0.82	0.83
D5S818	0.60	0.83	0.85	0.30	0.78	0.77	0.39	0.77	0.83	0.65	0.89	0.91
FGA	0.50	0.82	0.86	0.30	0.78	0.78	0.54	0.84	0.84	0.61	0.85	0.87
Amel	0.52	0.82	0.84	0.55	0.82	0.85	0.41	0.81	0.81	–	–	–

*Peak height ratios were determined for those heterozygous samples with peak heights greater than 100 RFU.
†Validation results in the AmpFℓSTR Identifiler® User's Manual (2001).
‡Median value.

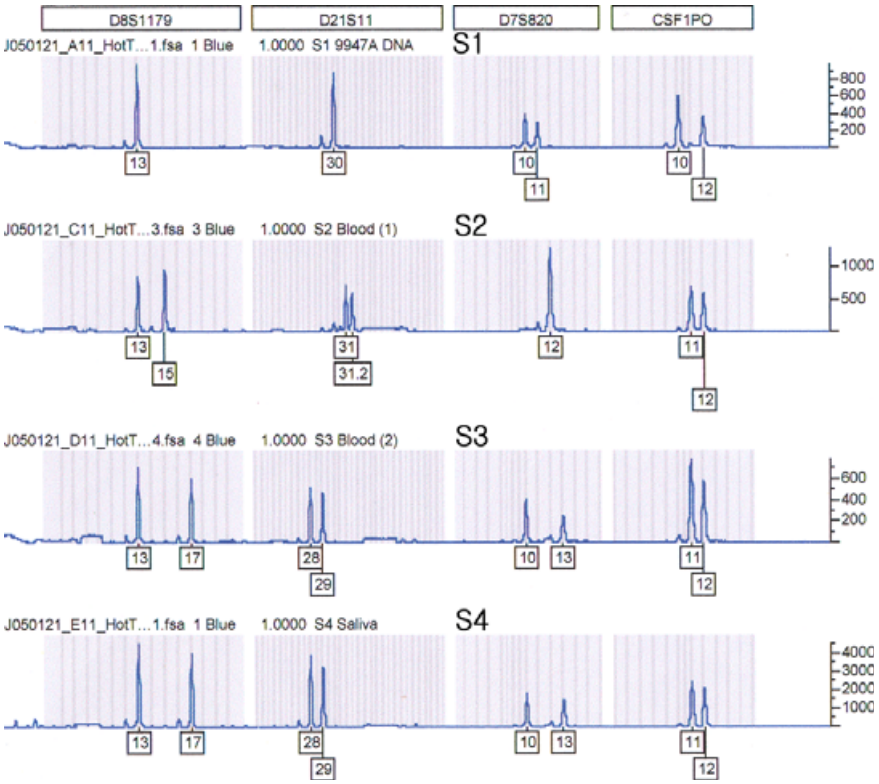

FIG. 4—*Comparison of direct PCR amplification of a 2-year-old blood spot with 9947A DNA and a fresh blood (saliva) spot. S1: control DNA (9947A); S2: 2-year-old blood spot; S3: fresh blood spot; S4: fresh saliva spot.*

purified DNA, all DNA profiles from blood and blood spots from 50 individuals matched fully. However, in the case of saliva spots, poor amplifications were sometimes observed, and this issue should be studied further. This system had broad compatibility with various DNA polymerase and STR kits. It is also supposed that direct PCR can be applied to old spots, which indicates that the system should be useful for fast DNA typing when there is no need to extract and store DNA, and when high-throughput is required. To be used actively in forensic case work, further validation studies are needed for various kinds of forensic stains and materials that might contain strong PCR inhibitory factors.

Acknowledgments

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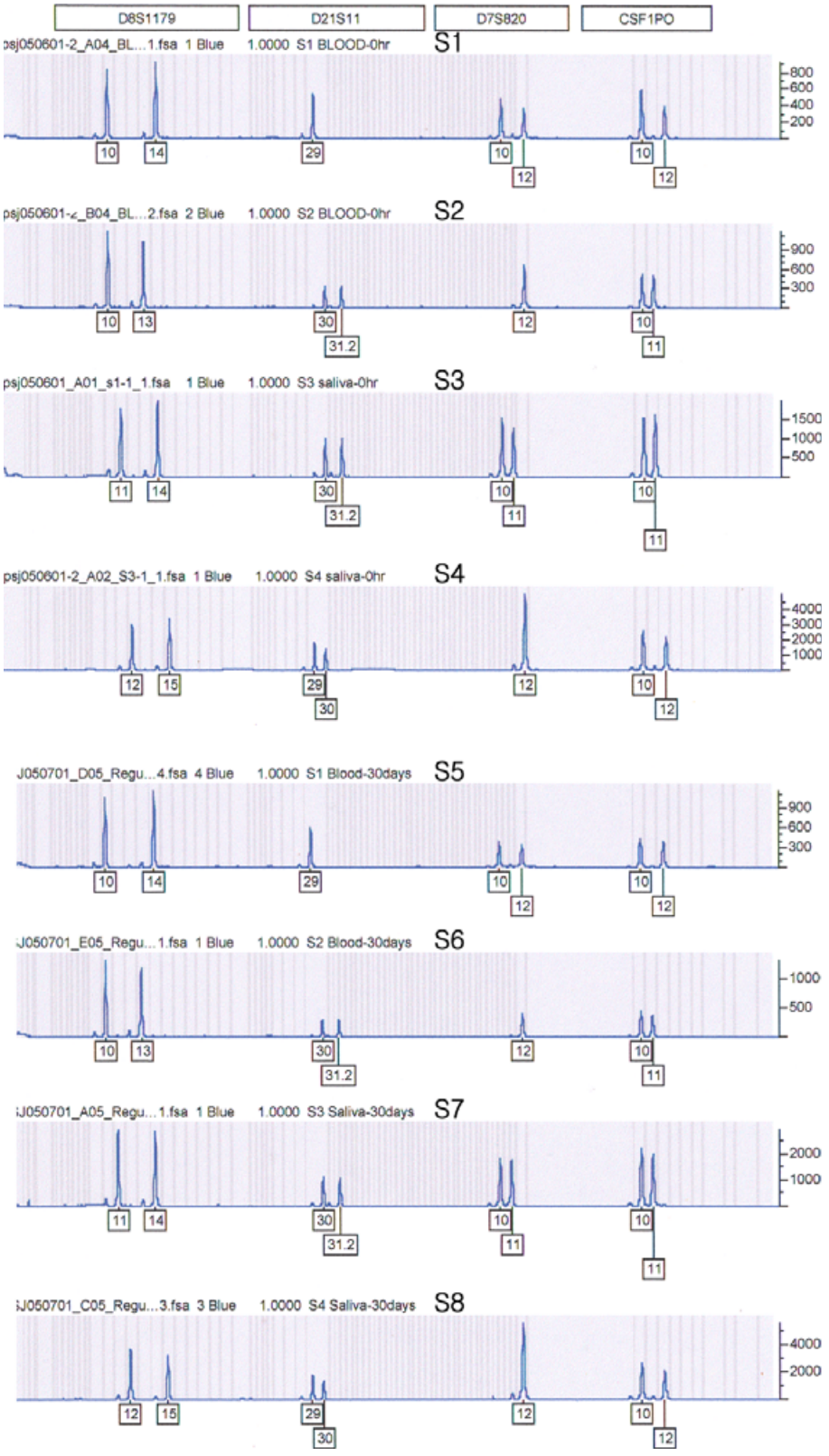

FIG. 5—*Time interval test of blood (or saliva) using direct PCR. S1–2: blood spot at 0 days; S3–4: saliva spot at 0 days; S5–6: blood spot after 1 month; S7–8: saliva spot after 1 month.*

TABLE 3—*Comparison of relative amplification qualities from blood spots when the primer concentration is reduced.*

No. of samples	Primer ratio	Identifiler*	Y filer†	Powerplex Y‡
$n = 9$	1§	+++¶	+++	+++
	1/2	+++	+++	++
	1/3	+	+	+/−**

*AmpFℓSTR Identifiler; †AmpFℓSTR Yfiler™; ‡Powerplex®Y; §concentration recommended by the manufacturer; ¶peak height and quality of complete DNA type; **allelic or locus drop-out observed.

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Additional information and reprint requests:
Seung Hwan Lee, Ph.D.
DNA Analysis Lab
Forensic Science Division, Supreme Prosecutors' Office
706, Banporo, Seocho-Gu
Seoul 137-730
Korea
E-mail: shlee@spo.go.kr