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Original Article

Validation of Reduced-volume Reaction in the PowerQuant® System for human DNA Quantification

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Since its introduction in the forensic field, quantitative PCR (qPCR) has played an essential role in DNA analysis. Quality of DNA should be evaluated before short tandem repeat (STR) profiling to obtain reliable results and reduce unnecessary costs. To this end, various human DNA quantification kits have been developed. Among these kits, the PowerQunat® System was designed not only to determine the total amount of human DNA and human male DNA from a forensic evidence item, but also to offer data about degradation of DNA samples. However, a crucial limitation of the PowerQunat® System is its high cost. Therefore, to minimize the cost of DNA quantification, we evaluated kit performance using a reduced volume of reagents (1/2-volume) using DNA samples of varying types and concentrations. Our results demonstrated that the low-volume method has almost comparable performance to the manufacturer's method for human DNA quantification, human male DNA quantification, and DNA degradation index. Furthermore, using a reduced volume of regents, it is possible to run 2 times more reactions per kit. We expect the proposed low-volume method to cut costs in half for laboratories dealing with large numbers of DNA samples.

Key Words: Quantitative PCR, Short tandem repeat (STR) profiling, PowerQunat® System, Human DNA quantification kit

INTRODUCTION

DNA profiling based on short tandem repeat (STR), which is characterized by high polymorphism and ease of genotyping, is the most popular method of human identification (Edwards et al., 1991; Hammond et al., 1994; Lygo et al., 1994; Budowle et al., 1999; Moretti et al., 2001). In essence, the STR profiling workflow for forensic casework samples consists of sample collection, DNA extraction, DNA quantification, STR amplification, capillary electrophoresis, and allele detection. The STR genotyping is sensitive to the quantity of DNA used in the PCR reaction (Lee et al., 2014). Therefore, reliable quantified human DNA makes it possible to adjust the concentration of template DNA used for STR analysis so that to obtain optimal PCR reactions and STR typing results with as small amounts of DNA as possible (Nielsen et al., 2008).

Quantitative PCR (qPCR) has played an essential role in DNA analysis since its first introduction in the forensic field (Westring et al., 2007; Ambers et al., 2016; Holt et al., 2016). It is a sensitive method for DNA quantification and

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Table 1. Types of case samples in this study

Samples	No. of samples
Postmortem Bone	
Femur	25
Costal cartilage	24
Spicule	5
Cranium	4
Teeth	1
Buccal cells/Saliva	12
Postmortem blood	8
Hair	4
Vaginal swab	2
Toothbrush	2
Razor	1
Total	88

is compatible with multiplexing targets. qPCR is routinely used to measure the concentration of total human and human male DNA (Alonso et al., 2003; Nicklas and Buel, 2003; Nielsen et al., 2008). Human DNA quantification kits have been developed to measure various sample conditions, such as DNA quantity, male DNA quantity, and the DNA degradation index (Hudlow et al., 2008; Vernarecci et al., 2015; Ewing et al., 2016). In order to obtain reliable results and reduce unnecessary costs, quality of DNA in terms of degradation should be evaluated before STR profiling.

The PowerQuant® System (Promega, Madison, WI, USA) combines multicopy quantification for each target and an internal PCR control (IPC) in one 5-dye qPCR assay to quantify total human DNA, human male DNA, and presence of degraded human DNA in a sample (Promega, 2015; Ewing et al., 2016). The PowerQuant® 2X Master Mix uses hot-start chemistry for the room temperature reaction set-up and is designed for performance comparable to newer STR systems (Thompson et al., 2013; Oostdik et al., 2014). In addition, the kit enables the quantification of DNA samples within 1 h. However, an important limitation of The PowerQuant® system in laboratories is its high cost. In order to improve cost efficiency of the kits, forensic DNA laboratories have been evaluating the applicability of commercial kits with reduced reagent volume. However, a low-volume method for PowerQuant® System is yet to be evaluated (Westring et al., 2007; Cho et al., 2018).

In the present study, we propose a low-volume method of PowerQuant® system and compare the results obtained using a reduced reagent volume (1/2) with the results obtained following the manufacturer's methods. We hypothesize that the low-volume method of the PowerQuant® system can perform comparably to the manufacturer's method. To test this prediction, we conducted a reproducibility test, PCR inhibitor test, and analysis of mixed DNA. Furthermore, we analyzed a total of 88 casework samples using the PowerQuant® system with a low-volume method to evaluate the practical applicability of the low-volume method.

MATERIALS AND METHODS

Sample preparation and DNA extraction

2800M control DNA (2800M), K562 genomic DNA (K-562) (Promega), AmpFℓSTR® Control DNA 9947A (9947A) and AmpFℓSTR™ DNA Control 007 (007) (Thermo Fisher Scientific, Waltham, MA, USA) were used as a standard DNA for evaluation of this study. To generate degraded DNA samples, control DNA samples (2800M and K562) were incubated at 98℃ for 15 min. All casework samples were obtained from the National Forensic Service in Korea. A total of 88 case samples, including postmortem bone, postmortem blood, saliva, vaginal swab and other sample types of forensic samples (Table 1). The DNA was isolated using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions (Qiagen, 2014).

DNA Quantification and Real-Time PCR method

DNA Quantification and assessment of DNA Degradation the human genomic DNA content of all samples was determined using the PowerQuant® System Kit (Promega) with the 7500 Real-time PCR System (Thermo Fisher Scientific). For all studies, serial 25-fold dilutions of the PowerQuant® Male gDNA Standard were amplified in duplicate to create four-point standard curves for the autosomal, Y, and degradation targets (Ewing et al., 2016). The standard curves were used to estimate a sample's DNA concentration based on the results of each target as described in the PowerQuant® System Technical Manual (Promega, 2015). Two types of

PowerQuant® system reaction volumes were used, the conditions recommended by the manufacturer (Total volume 18 μL) and a reduced volume (Total volume 9 μL). Samples were loaded into MicroAmp Optical 96-Well Reaction plates and sealed using Optical Adhesive Covers (Thermo Fisher Scientific). The thermal amplification conditions recommended by the manufacturer were used as follows: 98℃ for 2 min; 39 cycles of 98℃ for 15s and 62℃ for 35s.

STR Typing

Short tandem repeat analyses were performed using PowerPlex® Fusion System (Promega). A total 88 casework samples were amplified using ProFlex PCR System (Thermo Fisher Scientific) following the manufacturer's instructions (Oostdik et al., 2014). After STR amplification, 1 μL of amplicons was mixed with 16 μL of Hi-Di™ formamide (Thermo Fisher Scientific) and 0.16 μL of CC5 ILS 500 Size Standard (Promega). The mixture was subjected to capillary electrophoresis on the 24-capillary 3500xL Genetic Analyzer (Thermo Fisher Scientific) and analyzed using GeneMapper® ID-X v1.4 software (Thermo Fisher Scientific) with an analysis threshold of 100 relative fluorescent units (RFU) (Butler, 2012; Jung et al., 2020).

Reproducibility test

Multiple runs of both methods were performed with three different operators and two different 7500 Real-time PCR Systems and the results were compared as suggested in SWGDAM guidelines for validation of reproducibility. Two human genomic DNAs, which were 2800M (male DNA) and 9947A (female DNA), were prepared and quantified on each of three repeat runs (total 18 runs for each DNA).

Analysis of PCR inhibitor

Humic acid (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in distilled water for the PCR inhibitor test. The 2800M control DNA (1 ng/μL) containing 200~2,500 ng/μL of humic acid stock was prepared for the test. Samples were

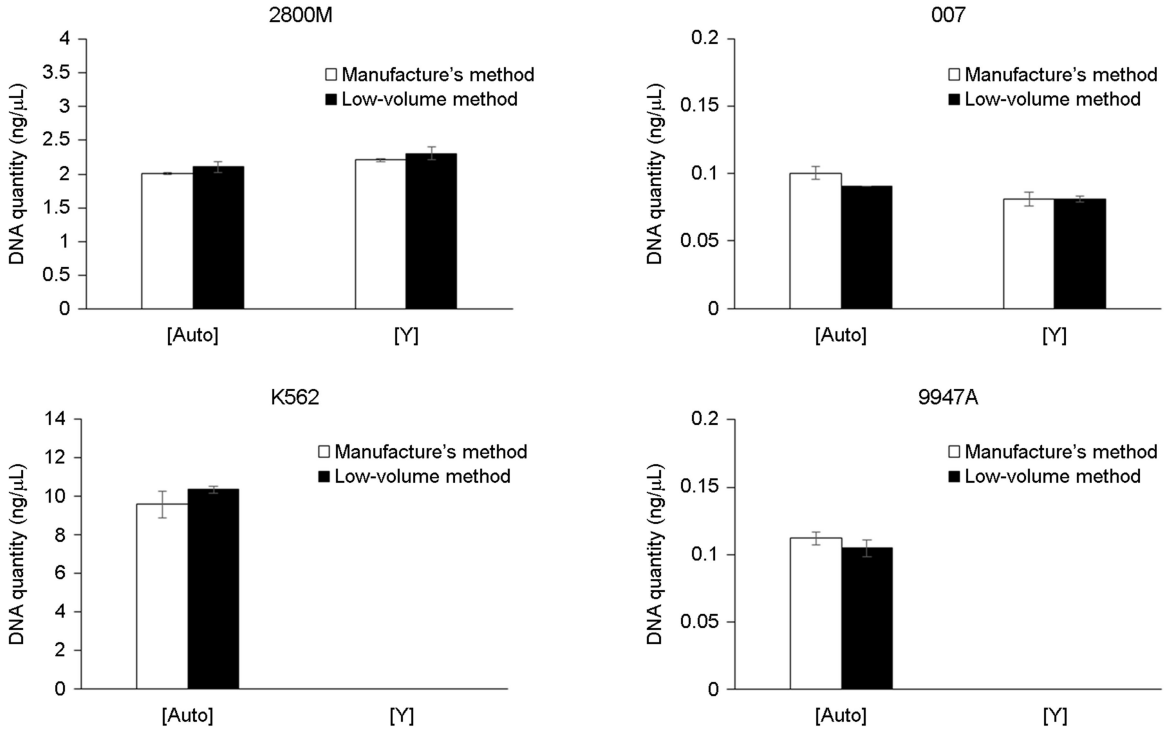

Fig. 1. Comparison between the manufacturer's method and the low-volume method using control DNA samples (2800M, 007, K562, 9947A) was performed using PowerQuant® System. All experiments were conducted three times. Error bar represents standard deviation.; Auto: autosomal DNA, Y: Y chromosomal DNA.

quantified in triplicate reactions using the PowerQuant® system following both the manufacturerμs method and the low-volume method.

Mixture analysis

Mixed DNA samples were prepared as a mixture of 2800M (male) and K562 (female) in a range of ratios from 1:1 to 1:500. The mixed DNA sample was quantified both in the manufacturer's method and the low-volume method.

RESULTS AND DISCUSSION

Comparison of the low-volume method to manufacturer' method regarding the quantification of control DNA samples

Four different kinds of control DNA samples with different concentrations were prepared, and the control DNA sample were quantified using PowerQuant® system according to the manufacturer's methods and the low-volume method (i.e., the 1/2 reduced use of reagents) to evaluate the consistency of the two different methods. The reactions were run in triplicate and the results from both methods were compared. To evaluate the reproducibility of the methods, coefficient of variation (CV) was calculated from triplicate experiments. For the autosomal DNA concentration of the control DNA samples, the CV values, as estimated using the manufacturer's method, ranged 0.6~7.2% (Fig. 1 and Table 2). The CV values, as estimated using the low-volume method, ranged 1.8~6.0% (Fig. 1 and Table 2). As a result of com paring both methods, the reproducibility of the low-volume method was as good as that of the manufacturer's method. We also evaluated the precision of the methods by calculating the root mean square error (RMSE) values. The precision of the low-volume method (RMSE = 0.007~0.393 ng/μL) was also comparable to that of the manufacturer's method (RMSE = 0.004~0.695 ng/μL) as shown in Table 2. For the male DNA concentration of the control DNA samples, the reproducibility and the precision of the low-volume method was also shown to be comparable to that of the manufacturer's method (Fig. 1 and Table 3). Our results have shown the consistency of the two methods and suggested comparable performance of the low-volume method to the manufacturer's method when quantifying the control DNA samples.

The PowerQuant® system includes the degradation target to determine the degree of DNA degradation which is useful to evaluate DNA quality in casework samples prior to experiments. The degradation target in the PowerQuant®

Table 2. Comparison of the manufacturer's method and the low-volume method for quantifying autosomal DNA concentration of control DNA samples

	Manufacturer's method			Low-volume method		
	Mean ± SD*	CV**	RMSE***	Mean ± SD	CV	RMSE
2800M (2 ng/μL)	2.009±0.011	0.6%	0.013	2.106±0.079	3.7%	0.124
007 (0.1 ng/μL)	0.100±0.005	5.0%	0.004	0.091±0.002	2.6%	0.009
K562 (10 ng/μL)	9.593±0.690	7.2%	0.695	10.36±0.188	1.8%	0.393
9947A (0.1 ng/μL)	0.112±0.004	3.7%	0.013	0.105±0.006	6.0%	0.007

*SD: standard deviation, **CV: coefficient of variation, ***RMSE: root mean square error

Table 3. Comparison of the manufacturer's method and the low-volume method for quantifying male DNA concentration of control DNA samples

	Manufacturer's method			Low-volume method		
	Mean ± SD*	CV**	RMSE***	Mean ± SD	CV	RMSE
2800M (2 ng/μL)	2.207±0.020	0.9%	0.207	2.304±0.096	4.2%	0.314
007 (0.1 ng/μL)	0.081±0.005	6.2%	0.019	0.081±0.009	10.7%	0.020

*SD: standard deviation, **CV: coefficient of variation, ***RMSE: root mean square error

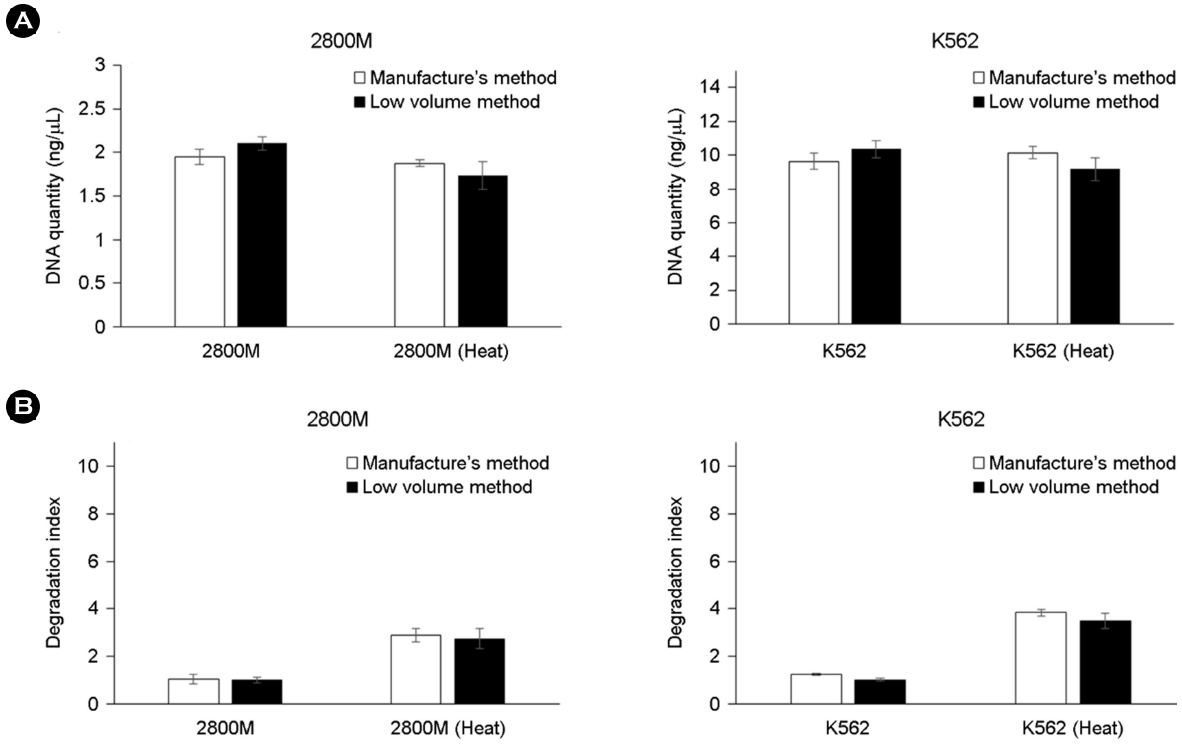

Fig. 2. Comparison of the manufacturer's method and the low-volume method for quantifying degraded female and male control DNA samples. The heated DNA 2800M and K562 (98℃ for 15 mins) were quantified using PowerQuant® System. Bar chart represents (A) DNA quantity and (B) degradation index. All experiments were conducted three times. Error bar represents standard deviation.

Table 4. Comparison of the manufacturer's method and the low-volume method for quantifying degraded control DNA samples

	Manufacturer's method					Low-volume method				
	Mean ± SD*	CV**	RMSE***	DI****	CV of DI	Mean ± SD	CV	RMSE	DI	CV of DI
2800M	2.009±0.011	0.6%	0.013	1.073±0.036	3.4%	2.106±0.079	3.7%	0.124	0.997±0.036	3.6%
2800M (degraded)	1.877±0.038	2.0%	0.331	2.883±0.192	6.7%	1.737±0.160	9.2%	0.705	2.736±0.134	4.9%
K562	9.593±0.690	7.2%	0.695	1.282±0.024	1.9%	10.36±0.188	1.8%	0.393	1.272±0.063	5.0%
K562 (hdegraded)	10.150±0.365	3.6%	0.332	3.834±0.149	3.9%	9.162±0.67	7.3%	1.002	3.487±0.327	9.4%

*SD: standard deviation, **CV: coefficient of variation, ***RMSE: root mean square error, ****DI: degradation index

system is 294 bp long which is longer than the autosomal (84 bp pair) and Y targets (81 bp and 136 bp), thus the degree of DNA degradation can be estimated by calculating autosomal target / degradation target ratio (Ewing et al., 2007). To further evaluate the performance of the low-volume method with degraded DNA, we prepared human control DNA samples which were degraded through exposure to 98℃ for 15 mins. In both methods, as expected, degradation index (DI, autosomal/degradation target ratio) values of the heat-degraded DNA samples increased compared to the non-degraded DNA samples, since the longer the target length is, the lower the PCR efficiency it gets in degraded DNA samples (Fig. 2 and Table 4). The CV values of the DI were lower than 10% in both manufacturer's method (1.9~6.7%) and low-volume method (3.6~9.4%). The autosomal DNA quantities of heat-degraded 2800M

Table 5. Analysis of samples mixed with male and female DNA

Samples	Expected rate (Auto/Y)	Manufacturer's method			Low-volume method		
		DNA quantity (ng/μL)		Rate (Auto/Y)	DNA quantity (ng/μL)		Rate (Auto/Y)
		Auto	Y		Auto	Y	
M1:F1	2	0.15	0.08	1.88	0.13	0.08	1.63
M1:F5	6	0.46	0.09	5.11	0.36	0.09	4.00
M1:F10	11	1.29	0.11	11.73	1.10	0.10	11.00
M1:F50	51	5.79	0.12	48.25	4.75	0.11	43.18
M1:F500	501	65.18	0.13	501.38	51.39	0.12	428.25

and heat-degraded K562 measured by low-volume method showed slightly low values (Fig. 2 and Table 4). CV and RMSE values from the low-volume method increased in heat-degraded samples than non-degraded samples. Moreover, the values were slightly higher than that obtained from the manufacturer's method (Fig. 2 and Table 4). Although both methods showed good reproducibility and precision, the performance of the manufacturer's method seemed to be slightly better when quantifying degraded DNA samples. However, the DI values estimated by the low-volume method was highly similar to that obtained by the manufacturer's method.

Quantification of mixed DNA sample with male and female DNA

To compare the performance of the methods regarding male and female DNA mixtures, we made mixed DNA samples of male and female DNA in a range of ratios from 1:1 to 1:500 (male:female) and quantified using both manufacturer's method and low-volume method. In our results, quantities of male DNA measured by both methods were almost similar, and autosomal DNA quantities measured by low-volume method appeared to be slightly lower than that measured by manufacturer's method (Table 5). The result from manufacturer's method showed that male to autosomal DNA rates (Auto/Y rate) were almost close to the expected values for all the mixture samples, while the Auto/Y rates from low-volume method turned out to be slightly lower than that from the manufacturer's method (Table 5). Although Auto/Y rates estimated by manufacturer's method seemed to be slightly more precise, our data suggest that the low-volume method had still good performance for quantifying male DNA in mixture samples.

Quantification of the casework DNA samples with low-volume method

Since there are various types of casework samples in forensics, it is important whether an analytical method is applicable to a wide range of sample types. To evaluate the performance of low-volume method, we firstly prepared five typical types of forensic casework samples (i.e., buccal swab, costal cartilage, vaginal swab, postmortem blood, and bone extract) and analyzed. The DNA quantities of the five different types of casework samples measured by low-volume method were highly similar to that by manufacturer's method (Fig. 3). The reproducibility and the precision of low-volume method were also highly similar to that by manufacturer's method indicating the good performance of low-volume method within a range of DNA concentrations (approximately 0.25~30 ng/μL) (Fig. 3).

We prepared 88 more casework samples and analyzed them to evaluate the applicability and performance of low-volume method in a wider variety of casework samples. The numbers and types of the samples are listed in Table 6. The types of the samples included postmortem blood, costal cartilage, cranium, femur, buccal swabs, saliva, hair, razor, spicule, teeth, toothbrush and vaginal swab, and they consisted of a wide range of concentrations (0.0031~19.0250 ng /μL). Both methods showed similar results overall in our casework study (Table 6). However, some of the results showed somewhat difference between the methods. Only a single run of experiment was performed in our casework

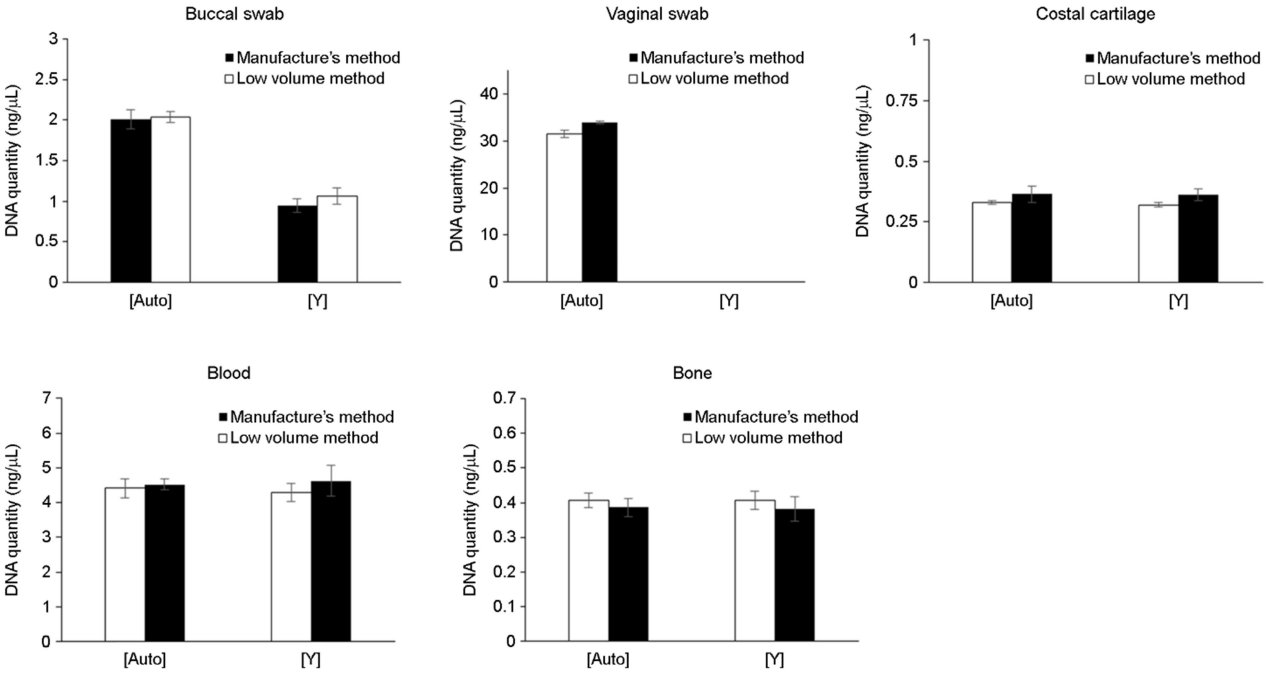

Fig. 3. Comparison of the manufacturer's method and the low-volume method using five casework samples (buccal swab, vaginal swab, costal cartilage, postmortem blood, postmortem bone). All experiments were conducted three times. Error bar represents standard deviation.; Auto: autosomal DNA, Y: Y chromosomal DNA.

Table 6. Comparative analysis of the manufacturer's method and the low-volume method with 88 case samples

		Manufacturer's method			Low-volume method			
		DNA quantity (ng/μL)			DNA quantity (ng/μL)			STR
No.	DNA source	Auto	Y	DI	Auto	Y	DI*	PPF**
01	Postmortem blood	**5.5063**	6.3402	1.2744	**5.4975**	6.3636	1.3300	F***
02	Postmortem blood	**2.8176**	2.3615	1.2996	**3.0237**	2.5416	1.3232	F
03	Postmortem blood	**5.7620**	Undet.	1.0315	**6.1918**	Undet.	1.1492	F
04	Postmortem blood	**7.6370**	9.7909	0.8761	**8.1176**	9.2700	0.9266	F
05	Postmortem blood	**3.2815**	4.0329	0.9519	**3.7772**	4.3422	1.0225	F
06	Postmortem blood	**19.0250**	20.4143	1.0613	**19.0862**	22.4279	0.9671	F
07	Postmortem blood	**3.0086**	2.7362	1.3195	**2.3555**	2.4048	1.1534	F
08	Postmortem blood	**0.0117**	0.0027	8.8436	**0.0054**	0.0020	6.5969	P(10/23)****
09	Costal cartilage	**1.3924**	1.8948	1.0630	**1.2546**	1.8383	0.9794	F
10	Costal cartilage	**0.8619**	0.8455	1.0242	**0.8187**	0.8032	1.0176	F
11	Costal cartilage	**0.7885**	0.9100	1.1026	**0.5615**	0.6617	0.9681	F
12	Costal cartilage	**1.0917**	1.0522	1.1299	**0.8057**	0.7901	1.0492	F
13	Costal cartilage	**0.4969**	0.5620	1.2002	**0.3286**	0.4125	1.1147	F
14	Costal cartilage	**1.1127**	0.9942	1.1674	**1.1270**	1.0185	1.1538	F
15	Costal cartilage	**0.5009**	0.0005	1.3381	**0.4968**	0.0005	1.2207	F
16	Costal cartilage	**0.5565**	0.5281	0.8658	**0.7005**	0.5520	0.9697	F
17	Costal cartilage	**1.6343**	1.9288	0.9453	**1.9741**	1.9305	1.0580	F
18	Costal cartilage	**1.4512**	Undet.	1.0423	**1.8958**	Undet.	1.1607	F

Table 6. Comparative analysis of the manufacturer's method and the low-volume method with 88 case samples (Continued)

		Manufacturer's method			Low-volume method			
		DNA quantity (ng/μL)			DNA quantity (ng/μL)			STR
No.	DNA source	Auto	Y	DI	Auto	Y	DI*	PPF**
19	Costal cartilage	**1.4279**	1.8390	1.0098	**1.6040**	1.8931	1.0312	F
20	Costal cartilage	**1.6052**	2.0105	0.9680	**1.7196**	2.1751	0.8733	F
21	Costal cartilage	**0.3842**	0.3820	1.0224	**0.3526**	0.3849	0.9542	F
22	Costal cartilage	**0.2644**	0.2564	1.1410	**0.2273**	0.2509	1.0927	F
23	Costal cartilage	**0.9637**	Undet.	1.4731	**0.9548**	Undet.	1.4602	F
24	Costal cartilage	**2.0120**	Undet.	1.2190	**2.0607**	Undet.	1.2064	F
25	Costal cartilage	**0.2602**	Undet.	1.1630	**0.3249**	Undet.	1.1116	F
26	Costal cartilage	**0.6793**	0.5888	1.4318	**0.8542**	0.7340	1.3518	F
27	Costal cartilage	**1.6330**	1.7314	1.3408	**1.9477**	1.9951	1.3738	F
28	Costal cartilage	**0.1018**	0.1090	1.4956	**0.1085**	0.1085	1.4043	F
29	Costal cartilage	**2.0996**	0.0010	1.1547	**2.4690**	0.0007	1.2448	F
30	Costal cartilage	**0.4219**	0.4760	1.0233	**0.5509**	0.5058	1.1106	F
31	Costal cartilage	**1.5216**	1.4541	1.1106	**2.5654**	2.6177	0.9861	F
32	Costal cartilage	**1.0330**	1.2426	1.1730	**1.0717**	1.1684	1.2817	F
33	Cranium	**0.0750**	0.0470	1.9142	**0.0676**	0.0593	1.8401	F
34	Cranium	**0.0242**	0.0012	2.7572	**0.0201**	0.0011	3.9065	F
35	Cranium	**0.2311**	0.1797	1.5587	**0.2166**	0.2051	1.4802	F
36	Cranium	**0.0041**	0.0010	5.3189	**0.0019**	0.0009	2.2020	P(1/23)
37	Femur	**0.0400**	0.0323	1.7344	**0.0220**	0.0227	1.4238	F
38	Femur	**0.3773**	Undet.	1.5100	**0.2627**	Undet.	1.4433	F
39	Femur	**0.0389**	0.0311	1.2577	**0.0326**	0.0352	1.1457	F
40	Femur	**0.3014**	0.2869	1.1186	**0.3042**	0.2889	1.2139	F
41	Femur	**0.0452**	0.0553	1.0941	**0.0529**	0.0572	1.3420	F
42	Femur	**0.1102**	0.0927	1.0930	**0.1187**	0.0967	1.4126	F
43	Femur	**0.0087**	0.0079	1.4927	**0.0120**	0.0100	2.3048	F
44	Femur	**0.0322**	0.0210	2.9652	**0.0293**	0.0169	4.0127	F
45	Femur	**0.1453**	Undet.	1.4611	**0.1666**	Undet.	1.8613	F
46	Femur	**0.2047**	Undet.	1.7456	**0.1380**	Undet.	2.6485	P(21/23)
47	Femur	**0.0329**	0.0252	1.3404	**0.0288**	0.0312	1.2842	F
48	Femur	**0.0031**	0.0034	2.2048	**0.0022**	0.0021	3.2720	F
49	Femur	**0.0297**	0.0224	1.2428	**0.0243**	0.0243	1.3131	F
50	Femur	**0.0340**	0.0205	2.6002	**0.0346**	0.0211	3.1455	F
51	Femur	**0.0336**	0.0314	1.1687	**0.0405**	0.0336	1.7112	F
52	Femur	**0.0262**	0.0188	2.9497	**0.0352**	0.0184	3.3224	F
53	Femur	**0.1054**	0.1075	1.2545	**0.1034**	0.0820	1.7333	F
54	Femur	**0.2488**	0.1923	2.1172	**0.1524**	0.1324	2.0247	F
55	Femur	**0.1303**	0.1146	1.7742	**0.1121**	0.1001	1.9151	F
56	Femur	**0.1185**	0.0738	3.8064	**0.0945**	0.0715	2.4155	F
57	Femur	**0.2297**	0.1513	1.7356	**0.1933**	0.1410	1.7705	F
58	Femur	**0.0161**	0.0115	1.8034	**0.0118**	0.0091	2.6972	P(20/23)

Table 6. Comparative analysis of the manufacturer's method and the low-volume method with 88 case samples (Continued)

		Manufacturer's method			Low-volume method			
		DNA quantity (ng/μL)			DNA quantity (ng/μL)			STR
No.	DNA source	Auto	Y	DI	Auto	Y	DI*	PPF**
59	Femur	**0.0295**	Undet.	2.5321	**0.0243**	0.0002	1.5776	F
60	Femur	**0.1522**	0.1068	1.8916	**0.1318**	0.1052	1.3666	F
61	Femur	**0.5335**	0.4941	2.1442	**0.5103**	0.4481	1.8157	F
62	Buccal cells	**1.6869**	Undet.	1.1686	**2.0733**	Undet.	1.1237	F
63	Buccal cells	**2.9327**	Undet.	1.0339	**2.6729**	Undet.	0.9922	F
64	Buccal cells	**0.4490**	0.4379	1.1812	**0.7113**	0.7589	1.0485	F
65	Buccal cells	**0.7585**	0.8157	0.9701	**1.2195**	1.3238	0.9042	F
66	Buccal cells	**0.7764**	Undet.	1.1406	**0.9710**	Undet.	0.9954	F
67	Buccal cells	**1.1101**	0.9777	1.1856	**1.5605**	1.3593	1.0882	F
68	Buccal cells	**0.1218**	Undet.	1.3193	**0.1827**	Undet.	1.1046	F
69	Buccal cells	**2.6815**	Undet.	1.4293	**3.8518**	Undet.	1.3235	F
70	Buccal cells	**0.5822**	0.5296	1.1385	**0.7522**	0.7236	1.0093	F
71	Buccal cells	**0.6674**	0.6946	1.2279	**0.9598**	0.9952	1.1265	F
72	Saliva	**0.4647**	Undet.	1.3889	**0.5032**	Undet.	1.1091	F
73	Saliva	**1.5625**	Undet.	1.4849	**2.0914**	Undet.	1.3294	F
74	Hair	**3.9015**	3.2693	1.7597	**3.0883**	3.1833	1.5757	F
75	Hair	**2.4082**	2.0729	1.2224	**2.0709**	2.1943	1.0722	F
76	Hair	**4.0924**	Undet.	1.2152	**4.0474**	Undet.	1.2099	F
77	Hair	**0.4009**	0.2922	1.8824	**0.3582**	0.3006	1.7153	F
78	Razor	**0.2341**	0.2356	1.2982	**0.2651**	0.2875	1.0921	F
79	Spicule	**0.0707**	0.0480	1.6700	**0.0688**	0.0492	1.8468	F
80	Spicule	**0.0850**	0.0781	1.4512	**0.1240**	0.0929	2.1957	F
81	Spicule	**1.3887**	1.0286	1.5551	**1.5103**	1.0837	1.8542	F
82	Spicule	**0.4574**	0.3045	1.9172	**0.3397**	0.2425	2.0708	F
83	Spicule	**0.2623**	0.2175	1.9713	**0.1570**	0.1584	1.8170	F
84	Teeth	**0.0220**	Undet.	2.2173	**0.0105**	Undet.	1.9815	F
85	Toothbrush	**0.5443**	0.6617	0.9884	**0.5787**	0.7429	0.8184	F
86	Toothbrush	**0.0445**	0.0434	1.5149	**0.0570**	0.0557	1.6800	F
87	Vaginal swab	**0.1342**	Undet.	2.4409	**0.1186**	Undet.	2.4080	F
88	Vaginal swab	**2.1518**	Undet.	2.4101	**2.5269**	Undet.	2.5370	F

*DI, Degradation index, **PPF, PowerPlex Fusion kit, ***F, Full profile, ****P, Partial profile

study because of the limited amount of casework DNA samples. We presumed that multiple runs of experiment would have reduced the gaps between the methods. In particular, the results obtained from buccal swabs showed the largest difference in the autosomal DNA quantity between the methods. It is suggested that 0.5~1 ng of DNA is optimal for STR analysis. Buccal swabs generally contain fresh and sufficient DNA that end up with good STR results, and we were able to obtain full-profiles in all the STR results of the buccal swabs (sample No. 61-71). Therefore, we consider that the difference between the methods in buccal swab samples to be acceptable and not to seriously affect STR results. When an unidentified body is found, postmortem blood can be the best specimen that leads to the best STR

Table 7. Results of inhibitor test using two methods of PowerQuant® system

	Manufacturer's method					Low-volume method				
	Inhibitor concentration (ng/μL)		Mean DNA quantity (ng/μL)			Inhibitor concentration (ng/μL)		Mean DNA quantity (ng/μL)		
	In sample	In reaction	Auto.	DI	Y	In sample	In reaction	Auto.	DI	Y
Control	0	0	1.05	0.93	1.15	0	0	1.10	1.15	1.28
HA-1	200	20	1.05	1.16	1.19	200	36	1.26	1.60	1.48
HA-2	400	40	1.04	1.28	1.25	400	73	1.20	1.64	1.56
HA-3	500	50	1.02	1.34	1.28	500	91	1.13	0.34	1.59
HA-4	1,000	100	0.83	0.10	1.20	1,000	182	Undet.	Undet.	Undet.
HA-5	2,000	200	0.28	Undet.	0.88	2,000	364	Undet.	Undet.	Undet.
HA-6	2,500	250	Undet.	Undet.	Undet.	2,500	455	Undet.	Undet.	Undet.

result if it is fresh. However, postmortem bloods are extremely vulnerable to decomposition. In our casework study, both methods concordantly showed that 7 of the postmortem blood samples had plenty of DNA, and the samples also showed full-profiles in STR results. In a postmortem blood which showed partial profile (10 out of 23) in the STR result, moreover, both methods concordantly indicated severe degradation of the DNA (sample No. 8). We need to use cartilages or bones when we cannot obtain blood. Cartilages are also decomposed as time goes by, and craniums and femurs remain in the end. Unfortunately, some DNA from cranium and femur samples show poor STR result. Compared to an undetected blood sample, one cranium sample (sample no.36) was degraded and had low DNA concentration to get STR profile, whereas two femur samples (sample no.46 and 58) were seemed to have sufficient DNA. In these femur samples DI index of low-volume method result was higher than manufacturer's method result. Therefore, it might be revealed that DI of low-volume method is more efficient for STR profiling. However, the quantification results of both methods were highly similar in the bone samples.

Evaluation of low-volume method with samples including PCR inhibitors

Samples were prepared with various concentrations of humic acid, which is a PCR inhibitor, and quantified by both methods and their results were compared. The samples contained 1 ng/μL of 2800M control DNA and 200~2,500 ng/μL of humic acid. In our results, PowerQuant® system was unable to determine the quantity of DNA when more than 2,500 ng/μL humic acid was included in the reaction in both methods (Table 7). Both methods were able to quantify DNA up to 500 ng/μL of humic acid (Table 7). The manufacturer's method was able to quantify the samples including 1,000~2,000 ng/μL humic acid while the low-volume method was not. However, the performance of the manufacturer's method also started to be inhibited by 1,000 ng/μL of humic acid. Amplification of degradation target was more sensitively affected by PCR inhibitor because of its long target sequence. In low-volume method, amplification of degradation target started to be inhibited from 500 ng/μL of humic acid, and manufacturer's method was inhibited from 1,000 ng/μL. In both methods, DNA volume was fixed to 2 μL while reagent volume was reduced in the low-volume method. In the manufacturer's method, therefore, the inhibitor is diluted 10-fold in the reaction mixture, while it is diluted 5-fold in the low-volume method. This explains why the manufacturer's method showed better performance with samples including inhibitor than the low-volume method. Although, there is a rare chance that casework samples include such high concentration of PCR inhibitors, the low-volume method seems to have more limitation quantifying the casework samples including high concentration of PCR inhibitors than the manufacturer's method.

Table 8. Summary of reproducibility test. (A) Average within run of three operators, (B) All runs variability of two control DNA

(A)	Average within-run variability											
	Mean DNA quantity (ng/μL)						Quant CV (%)					
	Manufacturer's method			Low-volume method			Manufacturer's method			Low-volume method		
Samples	Auto	DI	Y	Auto	DI	Y	Auto	DI	Y	Auto	DI	Y
9947-1	0.13	1.26	Undet.	0.13	1.27	Undet.	5.66	8.02	Undet.	6.97	9.63	Undet.
9947-2	0.13	1.18	Undet.	0.12	1.28	Undet.	5.28	11.71	Undet.	4.86	18.92	Undet.
9947-3	0.15	1.15	Undet.	0.15	1.41	Undet.	3.74	11.62	Undet.	6.74	13.18	Undet.
2800-1	0.91	0.97	1.08	0.87	1.06	0.89	2.83	13.48	3.44	9.17	11.26	3.85
2800-2	0.93	1.07	1.05	0.85	1.12	0.94	2.82	14.11	3.37	9.36	12.33	3.93
2800-3	1.06	0.99	1.13	0.82	0.99	0.99	4.31	10.31	5.88	8.92	14.41	5.00

(B)	All runs variability											
	Mean DNA quantity (ng/μL)						Quant CV (%)					
	Manufacturer's method			Low-volume method			Manufacturer's method			Low-volume method		
Samples	Auto	DI	Y	Auto	DI	Y	Auto	DI	Y	Auto	DI	Y
9947	0.14	1.20	Undet.	0.13	1.32	Undet.	10.67	10.65	Undet.	11.48	14.32	Undet.
2800M	0.97	1.01	1.09	0.85	1.06	0.94	7.88	12.76	5.26	8.95	12.92	5.96

Reproducibility of the low-volume method in multiple runs

Mean quantification results for autosomal, Y chromosomal and degradation targets of the six repeat runs (triple runs on each machine) by three different operators have been summarized in Table 8. Mean quantification result for each target was highly similar among the three different operators and between the methods (Table 8A). CV of the results from each operator (within-run) and 18 repeat runs from all the operators (all runs) were calculated in both methods to evaluate the reproducibility of the methods. Average within-run variability for 9947A samples looked similar between the methods overall. Average within-run variability for 2800M samples, in contrast, showed quite a large gap between the methods. CV values of autosomal target obtained from the low-volume method (9.17, 9.36 and 8.92%) turned out to be higher than those from the manufacturer's method (2.83, 2.82 and 4.31%), while CV values of degradation target from the low-volume method (11.26, 12.33 and 14.41%) were similar to those from the manufacturer's method (13.48, 14.11 and 10.31%). In addition, CV of all the 18 runs performed by three different operators were highly similar between the manufacturer's method and the low-volume method (Table 8B). The difference of CV values between the methods dramatically reduced in 18 runs because their SD values reached similar between the methods in 18 runs (data not shown). Based on our results, therefore, it is suggested that the low-volume method had similar reproducibility to the manufacturer's method.

DNA quantification reveals the quantity and quality of DNA in samples, which enables us to perform DNA analysis in the optimal experimental condition. The PowerQuant® system also lets us know the presence of male DNA and provides its quantity in samples, which is particularly important for the analysis of sexual assault cases. Therefore, DNA quantification helps us avoid unnecessary repetition of experiment and improves the quality of results. Large numbers of DNA analyses are performed in forensic DNA laboratories. As of 2019, a total of 332,096 DNA analyses were performed in the National Forensic Service in Korea. The introduction of DNA quantification dramatically reduced

reanalysis and minimized expenses, time, and consumption of samples. In the present study, we performed a comparative analysis of the manufacturer's method and the low-volume method with control DNA and casework DNA samples and found that the performance of the low-volume method is almost comparable to the manufacturer's method. We evaluated the low-volume method to reduce reagent consumption while maintaining performance. We expect that the proposed low-volume method will at least in part save costs in laboratories dealing with large numbers of DNA samples.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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