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Evaluation of two DNA extraction methods on exhumed bone samples: Ultrafiltration versus column affinity

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유골에서 DNA 추출법 비교 연구: Ultrafiltration과 Column affinity

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요 약: 발굴된 유골에서 DNA 추출은 DNA신원확인과정에서 매우 중요한 단계이지만 유골에 오염된 미생물 DNA와 사람 DNA가 동시에 추출되는 문제점이 있다. 이를 극복하기 위해 발굴된 10명의 유골 시료를 페놀-ultrafiltration 후 QIAquick® PCR Purification Kit (ultrafiltration법)와 QIAamp® DNA Mini kit(Column-affinity법)를 적용하여 전체(미생물+사람) DNA정량 및 사람 DNA정량 후 각각 STR마커를 분석하므로서 DNA 분리 효율성을 확인하였다. 회수된 전체 DNA양은 Column-affinity법(19.6 ng/μL)이 ultrafiltration법(16.0 ng/μL) 보다 1.2배 더 나은 결과를 보였으나 사람 DNA 정량 결과로는 Column-affinity법(0.034 ng/μL) 보다 ultrafiltration법(0.498 ng/μL)이 14.6배 더 많은 회수율을 나타냈다. 유골에 있던 다량의 미생물 DNA가 사람 DNA와 섞여있을 경우, 사람 DNA가 실리카 친화성 컬럼에 부착되는 것이 미생물 DNA의 영향을 받으나, 필터의 경우는 미생물 DNA의 영향을 받지 않아서 ultrafiltration법이 높은 회수율을 보였다.

Abstract: Extraction of DNA from skeletal material is of great importance in the identification of human remains, but is particularly difficult because the high amount of microbial DNA was often co-extracted with human bone DNA. We found that a phenol/chloroform extraction, followed by ultrafiltration, and cleanup by via the QIAquick® PCR purification kit yields higher amounts of human genomic DNA compared with extraction by the column affinity method® alone. Ultrafiltration extraction of human DNA from ten exhumed bone samples yielded 0.041–1.120 ng/μL DNA (mean = 0.498 ng/μL DNA), and purification using the column affinity resulted in 0.016–0.064 ng/μL DNA (mean = 0.034 ng/μL DNA). Although the STR genotyping by the column affinity method was partially successful, all DNA samples by the ultrafiltration method produced full profiles from the multiplex PCR. The efficiency of STR genotyping was in accordance with the amounts of the human DNA extracted.

Key words : forensic sciences, DNA extraction, bone, microbial DNA contamination

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1. Introduction

While investigating DNA extracted from bone, forensic and ancient DNA research has begun to focus more on the methodological problems associated with the extraction and analysis of DNA from bone samples recovered from mass graves, war remains, or forensic cases. This includes recent research on exogenous contamination,[1] problems with DNA degradation and damage,[2] and the possible co-extraction of PCR amplification inhibitors.[3,4] One less explored problem is the high amounts of microbial DNA that is often co-extracted with human DNA from the remains of exhumed bodies. Previous research has shown that DNA extracted from bone yields minimal amounts of degraded human DNA, mixed with high amounts of microbial DNA.[5-7] While most of the published techniques have been relatively effective in particular circumstances, it is difficult to predict which types of problems, cited above, will be encountered when working with any given sample.[8-12]

In this study, two DNA extraction methods were evaluated with respects to their ability to extract human specific DNA from exhumed bones: ultrafiltration versus column affinity. The efficiency of producing STR profiles from these extracts served as a measure of the quality of human DNA extracted by either method.

2. Materials and Methods

2.1. Bone samples

Ten bone specimens (femur shaft) excavated from several public cemeteries in South Korea, were selected for the study. Burial period of these human remains ranged from 1 year to 2 years. The bone fragments were stored at –70°C following their exhumation.

2.2. DNA extraction: Ultrafiltration based extraction

The bones were cleaned aggressively to remove the outer layer of foreign material. After crushing the bones into several pieces, they were placed in 50 mL conical tubes, decalcified in 20 mL of 0.5 M EDTA, pH 8.0, while being rocked gently at room temperature for one week. The bones were washed three times with distilled water to remove excess EDTA. After manually processing the bone material using a scalpel to obtain 1–2 mm bone particles, DNA was extracted from approximately 0.45 g of bone materials. Extraction-negative controls accompanied each set of extractions and were subjected to the following steps.

The samples were incubated overnight in 500 μL of lysis buffer at 56°C in water bath without shaking. The lysis buffer contained 10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 10 mM EDTA, 0.5% SDS, 39 mM dithiothreitol, and 0.4 mg/mL proteinase K. After the lysis, the samples were extracted using a phenol-chlorophorm protocol. DNA was first extracted by adding 500 μL of phenol/chloroform/isoamyl alcohol (25:24:1) to the lysate, and then centrifuged at 15000 rpm for 5 min. The aqueous phase was removed and subjected to a subsequent extraction using phenol/chloroform/isoamyl alcohol, as just described. The aqueous phase was concentrated with centrifugal filter devices with a Microcon ^{YM}-100 (Millipore, U.S.A) ultrafiltration membrane and each sample was eluted in 30 μL of distilled water. The extracted DNA was further purified with the QIAquick® PCR purification kit (Qiagen, Germany) in accordance with the manufacturer's instructions. DNA was dissolved in a total volume of 30 μL distilled water.

2.3. DNA extraction: Affinity based method

Parallel DNA extraction was performed on the same bone samples with column affinity method with the QIAamp® mini kit (Qiagen, Germany) in accordance with the manufacturer's instructions.

2.4. Total DNA and human DNA quantification

Since bacterial DNA may account for some of the total DNA recovered, the percentage of human DNA was determined for each extraction. The

concentration of total DNA extracted from bone was quantified by agarose gel electrophoresis and ethidium bromide staining. K562 DNA standards were used as a quantification standard from which a dilution series (40, 20, 10 and 5 ng of DNA) was generated. A standard curve from the DNA standards was generated and the DNA concentrations of the samples were extrapolated using this curve. The concentration of human DNA extracted from bone was determined with the Quantifiler® Human DNA Quantification Kit (Applied Biosystems) according to the manufacturer's protocol.

2.5. PCR amplification and analysis of STR profiles

DNA was amplified using the AmpFlSTR Identifiler kit (Applied Biosystems) in accordance with manufacturer's instructions (28 cycles). For the DNA samples extracted by ultrafiltration method, we performed additional amplification using the Powerplex 16 kit (Promega). Depending on the human DNA quantification results, the volume of template was adjusted to fit the manufacturer's recommendation on DNA concentration. The PCR products were separated using capillary electrophoresis with the 3100 Genetic Analyzer, Applied Biosystem and the results were analyzed by the GeneMapper ID software v3.1.

3. Results and Discussion

Considerably large amounts of human DNA were obtained using the ultrafiltration extraction method (*Table* 1). This is in stark contrast to the results of total DNA extracted where there was little difference between the yields of either method, and one extraction method did not consistently yield larger concentrations of DNA than the other. A wide variation of total DNA extracted from the different bone samples was observed. The total DNA concentration varied greatly between 0.3–38.0 ng/μL DNA (mean = 19.6 ng/μL DNA) by column affinity method, and 2.0–24.6 ng/μL DNA (mean = 16.0 ng/μL DNA) by ultrafiltration method. Column affinity yields of human DNA from bone samples ranged between 0.016–0.064 ng/μL DNA (mean = 0.034 ng/μL DNA). The ultrafiltration method gave adequate yields of human DNA from all bone tested, ranging between 0.041–1.120 ng/μL DNA (mean = 0.498 ng/μL DNA). Therefore, when microbial contaminated human DNA are extracted by column affinity method, extraction of sufficient quantities of human DNA for amplification becomes less likely, even extraction of total DNA is roughly similar with the results of the ultrafiltration method.

Since the column affinity extracted human DNA was of poor quantity, the STR profile results were

Table 1. Comparison of silica affinity and phenol/chloroform ultrafiltration methods of extracting DNA and their amount of total DNA and human DNA extracted

Bone samples	Amounts of total DNA (ng/μL)		Amounts of human DNA (ng/μL)	
	Column affinity	Ultrafiltration	Column affinity	Ultrafiltration
1	37.2	21.9	0.016	0.440
2	18.9	24.6	0.045	1.120
3	27.1	16.0	0.016	0.406
4	38.0	16.8	0.030	0.216
5	0.3	2.0	0.057	1.020
6	6.9	8.9	0.028	0.794
7	32.3	13.3	0.006	0.041
8	30.3	20.6	0.053	0.259
9	3.4	13.4	0.064	0.237
10	1.3	22.7	0.025	0.445
Mean	19.6 (1306.7 ng/g)	16.0 (1066.7 ng/g)	0.034 (2.3 ng/g)	0.498 (33.2 ng/g)

Table 2. Results of STR genotyping

Bone samples	AMEL	D19S433	D3S1358	D8S1179	D5S818	TH01	vWA	D21S11	D13S317	TPOX	D7S820	D16S539	D18S51	CSF1PO	D2S1338	FGA
1 (C)	XX	13 14	15 17	12 12	12 12	6 6	(17 17)		(12 12)	8 10		9 9				
1 (U)	XX	13 14	15 17	12 13	12 12	6 6	17 17	29 32.2	11 12	8 10	8 8	9 9	15 15	11 12	19 25	24 24
2 (C)	XY	13 14.2	15 16	13 14	11 12	6 9	18 20	29 30	10 12	8 11		9 11	(13 13)	(11 11)		
2 (U)	XY	13 14.2	15 16	13 14	11 12	6 9	18 20	29 30	10 12	8 11	11 12	9 11	13 13	11 11	18 19	21 24
3 (C)	XY	14 15.2	16 18	12 15	9 13	6 7		(29 29)		8 9		10 12		(12 12)	19 20	(22 24)
3 (U)	XY	14 15.2	16 18	12 15	9 13	6 7	18 18	29 31.2	10 12	8 9	11 11	10 12	14 14	12 12	19 20	22 24
4 (C)	(XY)	14 14		(16 16)	12 12		(16 17)			8 8						
4 (U)	XY	14 14	17 18	10 16	12 15	7 9	16 17	30 31	11 13	8 8	12 12	9 10	16 16	10 12	20 23	21 24
5 (C)	XY	13 14.2	15 16	13 16	10 11	9 9.3	14 18		(12 12)	8 9		9 12	15 15			(23 23)
5 (U)	XY	13 14.2	15 16	13 16	10 11	9 9.3	14 18	29 31.2	12 12	8 9	11 13	9 12	15 15	12 12	18 18	23 23
6 (C)	XY	15 15.2	15 15	13 13	10 11	7 9	17 17			9 11		11 13	13 13		20 22	(21 22)
6 (U)	XY	15 15.2	15 15	13 13	10 11	7 9	17 17	29 30	8 10	9 11	10 13	11 13	13 13	12 13	20 22	21 22
7 (C)	XY	14 14.2	15 17	(14 15)	12 12	6 9										
7 (U)	XY	14 14.2	15 17	14 15	12 12	6 9	14 18	28 32.2	8 11	8 11	10 11	9 10	13 15	9 11	18 26	19 21
8 (C)	XY	13 13.2	17 18	10 11	10 12	9 9	17 17	(31 32.2)	(8 11)	8 11		10 12		(12 12)		(20 21)
8 (U)	XY	13 13.2	17 18	10 11	10 12	9 9	17 17	31 32.2	8 11	8 11	11 12	10 12	14 21	12 12	17 20	20 21
9 (C)	XY	13 14	15 15	10 15	10 11	6 9	17 17	30 30	8 9	8 11	(8 11)	11 12	18 (20)	(10 12)	18 24	22 23
9 (U)	XY	13 14	15 15	10 15	10 11	6 9	17 17	30 30	8 9	8 11	8 11	11 12	18 20	10 12	18 24	22 23
10 (C)	XX	15 15.2	16 16	10 13	11 (12)	9 9	14 14	(30 31)	(8 9)	8 8		11 11	(14 19)	(12 12)		(21 22)
10 (U)	XX	15 15.2	16 16	10 13	11 12	9 9	14 14	30 31	8 9	8 8	9 11	11 11	14 19	12 13	18 19	21 22

C; Column affinity, U; Ultrafiltration. Alleles into brackets are inferior to 150 RFUs.

also poor. As expected, the STR typing efficiency was in accordance with the results of the yield of human DNA in the extracts. As shown in *Table* 2, we obtained full allelic profile for 1 bone and for 9 of them we detected only partial profiles composed of 6–14 loci out of a total of 16, respectively. However, in the case of the DNA extract with the ultrafiltration method, we obtained full allelic profiles of all 10 tested specimens. In the case of ultrafiltration method, we performed two different amplifications for each DNA extract with Identifiler and Powerplex 16. The results of these two allelic profiles were compared in order to determine the correct signals from the bone samples. Contamination arising from lab personnel could be ruled out in this case as each individual exhibits an STR profile unique from that exhibited by the bone samples.

Why does the ultrafiltration method work so much better than the column affinity? In addition to the QIAGEN® mini kit, we also applied the DNA IQ™ protocol for DNA extraction from these same samples, and obtained the similar poor results (data not shown). It has been reported that in the case of overwhelming microbial DNA, microbial DNA will compete with human DNA for binding to the silica resin with DNA IQ™ protocol; as a result, the human DNA yield will be poor.[13] In such case, it is recommended to increase the amount of silica resin to increase overall yield of total DNA. Such troubleshooting can be also applied to the QIAGEN® mini kit due to the fact that both methods are based on the same silica affinity, even though the exact character and quality of the silica materials used for DNA purification in the QIAGEN® mini kit is poorly defined.[14] Moreover, the negative influence of microbial DNA on human DNA binding was observed in a previous study.[15] It was reported that high amounts of microbial DNA could interfere with the specific hybridization of human sequences in a slot-blot format, rendering false negative results on the human DNA quantitation of bone and teeth DNA sample. In the case of ultrafiltration, Microcon YM-100 filters cut off the DNA by molecular weight, not by affinity. As such, the amount of human DNA retained by the Microcon ^{YM}-100 filter is not contingent on the amount of microbial DNA co-extraction from the same specimen. Moreover, further purification using QIAquick PCR purification kit has potential to remove not only degraded DNA fragments (<100 bp) but also the PCR inhibitor.[16]

In conclusion, modified ultrafiltration method was established on the microbial contaminated exhumed bone, showed higher yields of human genomic DNA and PCR product compared with affinity method. The modified ultrafiltration method can be the best choice for extracting human DNA from samples containing large amounts of microbial DNA.

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