Improved DNA extraction from old skeletal remains by combining total demineralization and silica-based extraction and concentration

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Introduction

A number of DNA extraction methods have been designed to obtain as much DNA as possible from old skeletal remains while minimizing the co-extraction of PCR inhibitors. Among many methods, a protocol which uses phenol/chloroform extraction followed by DNA concentration by a Centricon™ microconcentrator has been most widely used, but a protocol which uses a small-scale silica-based spin column has proven more efficient for the recovery of pure DNA. Recently, a complete demineralization method, which results in full physical dissolution of the bone samples, was proposed to maximize DNA yield, and a silica extraction method using a commercial large-scale kit was also reported to produce increased DNA yield while efficiently removing PCR inhibitors. In addition, the comparison of a number of ancient DNA extraction methods has led to the presentation of an optimized method which uses a buffer consisting solely of EDTA and proteinase K for bone digestion and binding of the DNA to silica via guanidinium thiocyanate for DNA purification. However, all of these recently proposed methods still have some drawbacks such as using phenol/chloroform extraction method, using Centricon™ microconcentrator for DNA concentration or using silica powders for DNA purification. Phenol/chloroform extraction and Centricon™ microconcentrator cannot efficiently remove potential PCR inhibitors, and silica powder itself is a powerful PCR inhibitor. In this report, we compared several DNA concentration/extraction methods using quantitative RT-PCR to assess DNA yield and removal of PCR inhibitors and present a simple and highly effective DNA extraction method for genotyping of skeletal remains that combines the most efficient methods from each step of the process.

Materials and Methods

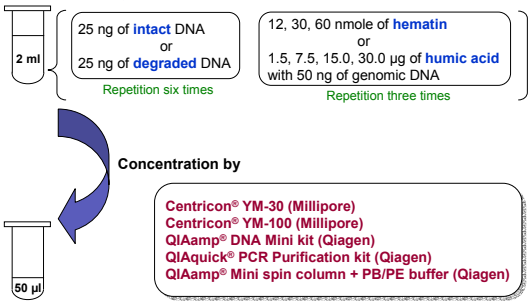
DNA Samples

Human genomic DNA (Promega, Madison, WI) to test several DNA concentration/extraction methods. Artificially degraded DNA was prepared by digesting 6.4 μg of human genomic DNA with 0.01 unit of DNase I for 30 min. To compare bone extraction methods, long bones from 55-year-old skeletal remains of 10 Korean War (1950-1953) victims were used.

Quantitative RT-PCR

Human genomic DNA content was determined using the Quantifiler™ Human DNA Quantification Kit and 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) with reduced-scale reactions consisting of 5 μl of Primer Mix, 6 μl of PCR Reaction Mix, and 1 μl of DNA extract in a final reaction volume of 12 μl.

DNA Recovery and Inhibitor Test



DNA Concentration from 2 ml of Sample to 50 μl

Ultrafiltration concentration		Silica-based column concentration		
Centricon® YM-30	Centricon® YM-100	QIAamp® DNA Mini kit	QIAamp® Mini spin column + PB/PE buffer	QIAquick® PCR Purification kit
2 ml of intact, degraded human genomic DNA solutions or inhibitors with genomic DNA solutions				
Ultracel YM-30	Ultracel YM-100	+ 2 ml of AL buffer/EtOH	+ 10 ml of PB buffer	
		QIAamp® Mini spin Column		QIAquick® spin column
Centrifuge 3500 x g	Centrifuge 2000 x g	Vacuum pump		
Twice with 2 ml of ddH_2O		750 μl of AW1/AW2 buffer	750 μl of PE buffer	
50 μl of ddH_2O				

DNA Extraction from Old Skeletal Remains

	Method A[a]	Method B	Method C
Demineralization	Incomplete	Complete	
Lysis buffer	15 ml of ATL buffer, 0.02 M DTT 10 mg Proteinase K	15 ml of 0.5 M EDTA, 0.5% SDS 3 mg Proteinase K	
1st Incubation	56°C, 48 hours		
2nd Incubation	+ 14 ml of AL buffer	+ 18 ml of AL buffer, 3 mg Proteinase K	+ 3 mg Proteinase K
	70°C, 1 hour		56°C, 1 hour
DNA recovery	Silica-based column extraction		
Column	QIAamp® Maxi spin column (Qiagen)		
Buffer	15 - 22 ml of EtOH 15 ml of AW1/AW2 buffer		75 ml of PB buffer 15 ml of PE buffer
Elution	2 ml of ddH_2O		
Concentration	QIAamp® Mini spin column + PB/PE buffer		

[a] Method A was based on Davoren et al. (Croat Med J. 2007:48;478-485) with substantial modifications using a QIAamp® Mini spin column with PB/PE buffers instead of using a Centriplus YM-100 column.

Results

Results of Quantitative RT-PCR Comparing Different DNA Concentration Methods Using Intact, Degraded Genomic DNA and Varied Concentrations of Hematin / Humic Acid

	Centricon® YM-30		Centricon® YM-100		QIAamp® DNA Mini kit		QIAamp® Mini spin column + PB/PE buffer		QIAquick® PCR Purification kit	
Amount[a] (per 2 ml)	Recovery[b] (%)	IPC C_T[c]	Recovery[b] (%)	IPC C_T[c]	Recovery[b] (%)	IPC C_T[c]	Recovery[b] (%)	IPC C_T[c]	Recovery[b] (%)	IPC C_T[c]
Human Genomic DNA (25 ng)										
Intact	10.2 ± 4.77	27.5 ± 0.08	7.0 ± 1.22	27.7 ± 0.15	34.7 ± 6.49	27.6 ± 0.11	38.9 ± 12.27	27.7 ± 0.09	27.3 ± 7.36	27.7 ± 0.09
Degraded	12.9 ± 6.22	27.6 ± 0.07	9.1 ± 4.51	27.7 ± 0.10	42.5 ± 9.32	27.5 ± 0.11	50.8 ± 12.24	27.4 ± 0.07	28.0 ± 11.06	27.6 ± 0.10
Hematin with DNA (50 ng)										
12 nmole	29.7 ± 5.22	27.9 ± 0.18	2.8 ± 0.12	27.6 ± 0.18	46.6 ± 6.11	27.6 ± 0.02	34.7 ± 6.27	27.6 ± 0.06	32.6 ± 9.36	27.6 ± 0.08
30 nmole	19.6 ± 3.27	29.6 ± 1.17	10.0 ± 4.73	27.8 ± 0.08	57.5 ± 3.06	27.7 ± 0.10	42.6 ± 9.04	27.7 ± 0.10	33.0 ± 8.80	27.8 ± 0.07
60 nmole	n.d.[d]	n.d.[d]	11.8 ± 0.68	27.9 ± 0.21	50.3 ± 16.41	27.6 ± 0.09	44.9 ± 12.18	27.6 ± 0.15	26.7 ± 2.59	27.8 ± 0.07
Humic acid with DNA (50 ng)										
1.5 μg	6.0 ± 4.06	27.6 ± 0.14	2.9 ± 0.59	27.6 ± 0.11	44.8 ± 7.05	27.5 ± 0.04	47.5 ± 3.70	27.5 ± 0.10	39.5 ± 16.90	27.5 ± 0.10
7.5 μg	7.5 ± 1.01	36.7 ± 3.09	7.6 ± 3.89	27.9 ± 0.13	39.7 ± 12.90	27.7 ± 0.17	49.5 ± 3.24	27.6 ± 0.05	38.9 ± 3.05	27.6 ± 0.13
15.0 μg	n.d.[d]	n.d.[d]	2.1 ± 0.58	27.9 ± 0.16	32.1 ± 14.66	27.7 ± 0.11	34.7 ± 7.46	27.5 ± 0.10	27.3 ± 7.00	27.6 ± 0.08
30.0 μg	n.d.[d]	n.d.[d]	3.8 ± 0.44	29.7 ± 0.94	18.5 ± 7.31	30.1 ± 1.56	23.8 ± 5.95	27.7 ± 0.19	17.3 ± 9.19	27.7 ± 0.11

[a] Amount in the initial DNA solutions, [b] DNA recovery rates (relative percentage) obtained by comparing concentrations of final DNA extracts with those of the initial DNA solutions without inhibitors, [c] Threshold cycles for log phase amplification of IPC, [d] not determined

Results of Quantitative RT-PCR Comparing Different DNA Extraction Methods for Old Skeletal Remains

		Method A		Method B		Method C	
Sample	Mass (g)	Concentration (pg/μl)	IPC C_T[a]	Concentration (pg/μl)	IPC C_T[a]	Concentration (pg/μl)	IPC C_T[a]
1	0.40	35.0 ± 3.42	28.3 ± 0.04	n.d.[c]	28.0 ± 0.00	114.8 ± 10.20	28.4 ± 0.02
2	0.50	n.d.[c]	27.8 ± 0.01	17.0 ± 3.64	27.8 ± 0.06	212.0 ± 6.19	28.1 ± 0.09
3	0.50	57.2 ± 0.80	28.2 ± 0.06	12.7 ± 1.12	27.8 ± 0.11	54.5 ± 7.86	28.1 ± 0.00
4	0.50	50.3 ± 7.51	27.5 ± 0.04	16.9 ± 3.11	27.5 ± 0.13	100.7 ± 4.62	27.5 ± 0.10
5	0.45	519.4 ± 37.82	27.9 ± 0.13	61.1 ± 4.89	27.7 ± 0.10	825.1 ± 44.46	28.0 ± 0.02
6	0.50	62.6 ± 14.43	28.0 ± 0.05	4.7 ± 3.86	27.8 ± 0.12	118.7 ± 32.71	28.3 ± 0.23
7	0.50	21.5 ± 3.12	27.7 ± 0.14	n.d.[c]	27.8 ± 0.12	34.4 ± 7.86	27.6 ± 0.08
8	0.55	143.2 ± 39.94	27.9 ± 0.19	28.9 ± 0.44	28.0 ± 0.11	156.8 ± 7.55	28.4 ± 0.02
9	0.50	54.9 ± 2.70	28.3 ± 0.11	29.1 ± 10.71	28.6 ± 0.07	112.4 ± 24.15	28.6 ± 0.23
10	0.50	70.7 ± 3.58	28.4 ± 0.11	35.7 ± 1.55	27.9 ± 0.33	327.3 ± 16.58	28.7 ± 0.11
Degraded DNA[b]	5.0×10^{-8}	232.4 ± 73.41	27.5 ± 0.14	91.0 ± 20.27	27.6 ± 0.05	236.8 ± 77.32	27.7 ± 0.15

[a] Threshold cycles for log phase amplification of IPC, [b] Artificially degraded genomic DNA substituted for bone powder, [c] not determined

Conclusion

- **The silica-based column was much more efficient at recovering and concentrating intact and degraded genomic DNA. Especially, the QIAamp® Mini spin column combined with buffer PB/PE from the QIAquick® PCR Purification kit showed the highest recovery of degraded DNA.**
- **IPC assay by RT-PCR using various concentrations of inhibitors showed that almost all methods had good inhibitor removal, but ultrafiltration using the Centricon® YM-30 yielded undetermined C_T values at high inhibitor concentrations. At the concentration of 30.0 μg of humic acid in 2 ml, both Centricon® YM-100 and the QIAamp® DNA Mini kit showed they could not sufficiently remove inhibitors. The method using QIAamp® Mini spin column combined with buffer PB/PE from the QIAquick® PCR Purification kit was most efficient both at recovering DNA and at removing inhibitors.**
- **When comparing the DNA extraction methods (A,B and C) for old skeletal remains, method C consisting of complete demineralization followed by a combined DNA extraction procedure using QIAamp® Maxi spin columns and buffer PB/PE from the QIAquick® PCR Purification kit showed the best performance.**