Preliminary DNA Identification for the Tsunami Victims in Thailand

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The 2004 Southeast Asia Tsunami killed nearly 5,400 people in Southern Thailand, including foreign tourists and local residents. To recover DNA evidence as much as possible from the seriously decomposed bodies, we explored procedures of sample preparation from both bone and tooth samples as well as both mitochondrial and nuclear markers. Despite having failed to recover enough DNA for nuclear marker typing, we succeeded in obtaining fully informative results for mitochondrial markers (HV1 and HV2) from 258 tooth samples with a success rate of 51% (258/507). Using an organic DNA extraction method coupled with an ultrafiltration step, we obtained 16 STR (including 13 CODIS loci, one sex discrimination locus, and two Identifiler loci) profiles for 834 samples with a success rate of 79% (834/1,062). In addition, by comparing the allelic frequencies between the typed samples as a group and other index populations, we conclude that the Thai tsunami victims are a combined group of several populations. Our results provide valuable evidence and protocols for the future forensic practice.

Key words: DNA identification, decomposed body, tsunami victims, mitochondrial marker

Introduction

Brought by an Indian Ocean earthquake, the notorious 2004 Southeast Asia Tsunami killed nearly 5,400 people in Southern Thailand. The victims include a large number of foreign tourists from Europe, Asia, and other regions of the world, in addition to Thai nationals. As part of an international relief effort, we participated in a scientific and humane endeavor to reveal the identity of these victims based on current forensic methods with important modifications.

The Chinese scientists arrived in Phuket, Thailand on December 31, 2004, and joined immediately the multi-national task force to collect samples from the remains of victims. Due to the scale of the disaster, the climate, and the process to initiate an adequate rescue effort, by the time when large-scale sample collection initiated, the remains of most tsunami victims had been seriously decomposed, not only making the sample collection process very difficult but also posing questions about the success of DNA identification. After in-depth literature studies and discussions with experts in this field, on January 1, 2005, we made a critical proposal that the samples collected for forensic tests should definitely include thick bones in addition to teeth, although the latter are the preferred specimens by standard forensic and anthropological studies for DNA-based identification. We have three basic arguments. First, the decomposing process in a humid and high-temperature environment may be significantly accelerated, especially when plentiful ocean-borne microbial species are stirred up by tsunami waves from oceanic sediments. Second, teeth should be among the first body parts being exposed to the microbe-rich seawater and the situation should be worsened when the floating bodies, often facedown, are soaked for days. Third, DNA-rich bone marrows are covered by the skin, muscle tissues, and calcified compact bones so that it takes much longer time for

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microbes to penetrate through. Upon the acceptance of our proposal by the task force, 507 tooth samples and 1,062 bone (a portion of femur) samples were collected on site by certified forensic dentists and forensic anthropologists, respectively, and were received by our laboratory in Beijing, from January 15 to June 11, 2005.

In this report, we describe our experience in DNA identification and results from a preliminary analysis on two types of data from nuclear and mitochondrial markers, which were acquired from two different specimens, teeth and bones. These results and analyses are believed to be highly beneficial for forensic scientists who may handle samples from specific environmental conditions, not limited to tsunami victims.

Results

Genotyping of the HV1 and HV2 loci with mitochondrial DNA from tooth specimens

Since most of the specimens from teeth had failed in STR typing due to low abundance and degradation of the nuclear DNA, we used these samples for mitochondrial DNA (mt-DNA) sequence analysis. We have succeeded in obtaining results from 258 tooth samples (258/507, success rate 51%) in both HV1 and HV2 sequences. A comparison to the Anderson sequences and results are summarized in Tables 1 and 2.

Table 1 Statistics of 258 Tooth Samples from HV1 and HV2

Locus*	S#(Ts/Tv)	No.	Ins.	No.	Del.	No.	Freqency	Locus*	S#(Ts/Tv)	No.	Ins.	No.	Del.	No.	Freqency
\-	\-	Statistics of 258 tooth samples from HV1						\-	\-	Statistics of 258 tooth samples from HV1					
16004	C-T	10					1.13%	16131	T-G	1					0.11%
16019	C-T	16					1.81%	16134	C-T	2					0.23%
16021	C-T	4					0.45%	16136	T-C	2					0.23%
16026	C-T	23					2.60%	16136	T-A	1					0.11%
16030	C-T	2					0.23%	16140	T-C	2					0.23%
16032	T-A	3					0.34%	16140	T-A	2					0.23%
16032	T-G	2					0.23%	16142	C-A	1					0.11%
16042	G-A	1					0.11%	16145	G-A	9					1.02%
16051	A-G	6					0.68%	16148	C-T	1					0.11%
16053					C	2	0.23%	16149	A-C	1					0.11%
16067	C-T	1					0.11%	16153	G-A	5	C		6		1.25%
16069	C-T	21					2.38%	16154	T-C	1					0.11%
16070	A-G	1					0.11%	16157	T-A	1					0.11%
16085	C-G	3					0.34%	16159	C-A	1					0.11%
16085	C-A	1					0.11%	16162	A-G	16					1.81%
16086	T-C	4					0.45%	16163	A-G	4					0.45%
16092	T-C	7					0.79%	16167	C-T	1					0.11%
16093	T-C	7					0.79%	16169	C-T	2					0.23%
16095	C-T	1					0.11%	16171	A-G	1					0.11%
16095	C-G	2					0.23%	16172	T-C	20					2.27%
16104	C-T	1					0.11%	16174	C-T	3					0.34%
16104	C-A	1					0.11%	16176	C-T	1					0.11%
16108	C-T	5					0.57%	16176	C-G	1					0.11%
16111	C-T	3					0.34%	16179	C-T	1					0.11%
16114	C-A	4					0.45%	16182	A-C	13					1.47%
16124	T-C	2					0.23%	16183	A-C	28					3.17%
16126	T-C	46					5.21%	16186	C-T	3					0.34%
16127	A-C	1					0.11%	16187	C-T	2					0.23%
16129	G-A	28					3.17%	16188	C-T	1					0.11%
16129	G-C	2					0.23%	16189	T-C	65					7.36%

Table 1 *Continued*

Statistics of 258 tooth samples from HV1								Statistics of 258 tooth samples from HV1							
Locus*	S#(Ts/Tv)	No.	Ins.	No.	Del.	No.	Freqency	Locus*	S#(Ts/Tv)	No.	Ins.	No.	Del.	No.	Freqency
16192	C-T	15					1.70%	16287	C-T	2					0.23%
16193	C-T	2	C	3			0.57%	16288	T-C	1					0.11%
16195	T-C	1					0.11%	16290	C-T	2					0.23%
16203	A-G	2					0.23%	16291	C-T	7					0.79%
16207	A-G	2					0.23%	16292	C-T	10					1.13%
16209	T-C	4					0.45%	16293	A-G	5					0.57%
16213	G-A	5					0.57%	16293	<u>A-C</u>	3					0.34%
16217	T-C	5					0.57%	16293	<u>A-T</u>	1					0.11%
16218	C-T	2					0.23%	16294	C-T	27					3.06%
16219	A-G	1					0.11%	16295	C-T	2					0.23%
16221	C-T	4					0.45%	16296	C-T	12					1.36%
16222	C-T	4					0.45%	16297	T-C	2					0.23%
16223	C-T	53					6.00%	16298	T-C	19					2.15%
16224	T-C	10					1.13%	16301	C-T	1					0.11%
16227	A-G	1					0.11%	16303	G-A	1					0.11%
16230	A-G	1					0.11%	16304	T-C	24					2.72%
16231	T-C	8					0.91%	16305	<u>A-T</u>	1					0.11%
16232	C-T	1					0.11%	16309	A-G	6					0.68%
16232	<u>C-A</u>	3					0.34%	16311	T-C	28					3.17%
16233	A-G	2					0.23%	16319	G-A	3					0.34%
16234	C-T	3					0.34%	16319	<u>G-C</u>	1					0.11%
16235	A-G	2					0.23%	16320	C-T	1					0.11%
16239	C-T	3					0.34%	16324	T-C	2					0.23%
16240	A-G	2					0.23%	16325	T-C	4					0.45%
16242	C-T	1					0.11%	16326	A-C	1					0.11%
16243	T-C	2					0.23%	16327	C-T	4					0.45%
16247	A-G	1					0.11%	16335	A-G	3					0.34%
16249	T-C	4					0.45%	16342	T-C	1					0.11%
16255	G-A	1					0.11%	16343	A-G	1					0.11%
16256	C-T	13					1.47%	16344	C-T	1					0.11%
16257	C-T	1					0.11%	16351					A	1	0.11%
16258	A-G	1					0.11%	16353	C-T	1					0.11%
16260	C-T	5					0.57%	16354	C-T	2					0.23%
16261	C-T	16					1.81%	16355	C-T	1					0.11%
16263	T-C	5					0.57%	16356	T-C	8					0.91%
16265	A-G	1					0.11%	16360	C-T	1					0.11%
16266	C-T	4					0.45%	16361	<u>G-C</u>	2					0.23%
16266	<u>C-A</u>	1					0.11%	16362	T-C	29					3.28%
16269	<u>A-C</u>	1					0.11%	16373	G-A	5					0.57%
16270	C-T	21					2.38%	16381	T-C	2					0.23%
16271	T-C	1					0.11%	16384	G-A	3					0.34%
16278	C-T	13					1.47%	16390	G-A	6					0.68%
16286	C-T	2					0.23%	16391	G-A	4					0.45%
16286	<u>C-A</u>	1					0.11%	Total		871		9		3	100%(883)

Table 1 *Continued*

Locus*	S#(Ts/Tv)	No.	Ins.	No.	Del.	No.	Freqency	Locus*	S#(Ts/Tv)	No.	Ins.	No.	Del.	No.	Freqency
							Statistics of 258 tooth samples from HV2								Statistics of 258 tooth samples from HV2
46	T-A	1					0.08%	242	C-T	2					0.17%
61	C-A	1					0.08%	249					A	19	1.59%
61	C-T	12					1.00%	250	T-C	3					0.25%
64	C-T	20					1.67%	253	C-T	1					0.08%
72	T-C	7					0.58%	253	C-G	1					0.08%
72	T-G	5					0.42%	257	A-G	1					0.08%
73	A-G	118					9.86%	259	A-G	1					0.08%
93	A-G	2					0.17%	262	C-T	1					0.08%
94	G-A	2					0.17%	263	A-G	249					20.80%
114	C-T	1	C	3			0.33%	264	C-T	1					0.08%
132	C-T	3					0.25%	282	T-C	1					0.08%
140	C-T	1					0.08%	285	C-T	2					0.17%
143	G-A	3					0.25%	290					AA	2	0.17%
146	T-C	20					1.67%	291	A-T	1					0.08%
150	C-T	27					2.26%	295	C-T	22					1.84%
151	C-T	2					0.17%	297	A-G	1					0.08%
152	T-C	46					3.85%	309			C	101			8.44%
153	A-G	3					0.25%	309			CC	24			2.01%
173	T-C	1					0.08%	310	T-C	4					0.34%
183	A-G	7					0.58%	310			C	21			1.75%
185	G-A	7					0.58%	310			TC	6			0.50%
188	A-G	3					0.25%	315			C	200			16.79%
189	A-G	13					1.09%	315			CCC	1			0.08%
192	T-C	1					0.08%	316	G-C	1					0.08%
194	C-T	9					0.76%	317	C-T	1					0.08%
195	T-C	47					3.93%	317	C-G	1					0.08%
196	T-C	1					0.08%	319	T-C	6					0.50%
198	C-T	2					0.17%	323	G-A	1					0.08%
199	T-C	8					0.67%	345	C-T	1					0.08%
200	A-G	2					0.17%	356			C	2			0.17%
204	T-C	17					1.42%	362	C-A	22					1.84%
207	G-A	16					1.34%	366	G-A	11					0.92%
210	A-G	1					0.08%	376	A-C	1					0.08%
214	A-G	4					0.34%	379	A-C	2					0.17%
215	A-G	7					0.58%	380	G-C	3					0.25%
215	A-C	1					0.08%	385	A-G	1					0.08%
217	T-C	4					0.34%	389	G-A	1					1.01%
222	C-T	1					0.08%	402	A-T	1			A	1	0.17%
225	G-A	5					0.42%	404	C-T	2					0.17%
226	T-C	2					0.17%	408	T-G	3					0.25%
227	A-G	2					0.17%	411	C-G	4					0.33%
228	G-A	11					0.92%	463			C	1			0.08%
234	A-G	2					0.17%	Total		816		356		25	100.00%
239	T-C	4					0.34%								(1,197)

*Locus lists the base location in the human mitochondrial DNA (D-loop region). #S stands for substitutions. The detailed base changes, transition (Ts) or transversion (Tv) (underlined), are listed.

Table 2 Alleles and Their Frequencies in 15 Autosomal STR Loci

Locus	Allele and Frequency												
CSF1PO	7	0.2635%	8	0.3953%	9	2.5692%	10	25.2964%	11	30.3689%	12	34.8485%
	13	5.4677%	14	0.7246%	15	0.0659%						
FGA	16	0.3157%	17	0.3157%	18	1.5152%	19	6.6919%	20	6.2500%	20.2	0.2525%
	21	14.8990%	21.2	1.8308%	22	20.5177%	22.2	1.1995%	23	16.4773%	23.2	1.0101%
	24	12.5000%	24.2	0.9470%	25	9.1540%	25.2	0.6313%	26	3.7247%	26.2	0.1894%
	27	1.1364%	28	0.4419%								
TH01	4	0.1238%	6	12.0050%	7	25.8045%	8	8.4777%	8.3	0.1238%	9	37.0050%
	9.3	10.3342%	10	6.0644%	11	0.0619%						
TPOX	6	0.1845%	8	56.3961%	9	10.6396%	10	3.1365%	11	27.9213%	12	1.5990%
	13	0.1230%										
vWA	13	0.1230%	14	21.4637%	15	4.3665%	16	15.1292%	17	27.4293%	18	19.8032%
	19	9.4711%	20	2.0295%	21	0.1230%	22	0.0615%				
D3S1358	12	0.2466%	13	0.3083%	14	5.9186%	15	28.7916%	16	33.4772%	17	23.2429%
	18	7.3366%	19	0.5549%	20	0.1233%						
D5S818	7	1.6049%	8	0.0617%	9	5.5556%	10	22.5926%	11	26.6667%	12	24.5062%
	13	17.6543%	14	1.2346%	15	0.1235%						
D7S820	7	1.3871%	8	19.5509%	9	8.3884%	10	15.9181%	11	34.2140%	12	17.3052%
	13	2.9062%	14	0.3303%								
D8S1179	8	0.4305%	9	0.1845%	10	13.6531%	11	8.0566%	12	11.9311%	13	18.5732%
	14	18.6347%	15	19.5572%	16	7.5031%	17	1.2915%	18	0.1845%		
D13S317	6	0.0634%	7	0.1901%	8	28.7072%	9	12.4842%	10	9.8859%	11	23.0672%
	12	18.3143%	13	5.7034%	14	1.5843%						
D16S539	8	0.9963%	9	18.8045%	10	10.3362%	11	31.9427%	12	25.1557%	13	11.0834%
	14	1.5567%	15	0.0623%	16	0.0623%						
D18S51	7	0.0650%	9	0.2601%	10	0.4551%	11	1.1704%	12	5.9818%	13	14.3043%
	13.2	0.2601%	14	18.4655%	14.2	0.4551%	15	22.9519%	16	15.6047%	17	7.9974%
	18	4.4863%	19	2.8609%	20	0.9103%	21	1.6905%	22	1.3004%	23	0.3901%
	24	0.2601%	25	0.1300%								
D21S11	27	0.4969%	28	8.0745%	28.2	0.1863%	29	24.6584%	29.2	0.3106%	30	24.9068%
	30.2	3.2298%	31	6.7702%	31.2	8.0124%	32	2.4224%	32.2	14.1615%	33	0.1863%
	33.2	5.7764%	34.2	0.6832%	35.2	0.1242%						
D2S1338	16	1.4916%	17	12.9053%	18	10.0519%	19	18.6770%	20	11.2840%	21	3.9559%
	22	5.8366%	23	16.6667%	24	12.4514%	25	5.9014%	26	0.7782%		
D19S433	9	1.0481%	11	0.0617%	11.2	0.2466%	12	5.2404%	12.2	0.4932%	13	25.4624%
	13.2	4.5623%	14	26.2639%	14.2	8.7546%	15	8.8163%	15.2	14.3033%	16	1.7879%
	16.2	2.5277%	17	0.0617%	17.2	0.2466%	18.2	0.1233%				

In the HV1 region, we detected 147 loci from base 16004 to 16391 in a total of 883 variants, including 871 SNPs and 12 Indels (Insertions and Deletions). There are eight loci with frequencies exceeding 3% (16126, 16129, 16183, 16189, 16223, 16294, 16311, and 16362). In the HV2 region, we found 85 loci from base 46 to 463 with a total of 1,197 variants. Among them, we have 816 SNPs and 381 Indels. There are four high-frequency loci in this region (73, 263, 309, and 315). We notice the uneven distributions of the variants be-tween the two regions among the loci in their distri-bution, allelic frequencies, and variation types (base substitutions and Indels).

Genotyping of nuclear STR loci with nuclear DNA from bone specimens

After encountering difficulty in extracting high-quality and adequate DNA from tooth specimens, we tried three different methods to extract DNA from bone specimens. The IQ™ system from Promega

and the QIAamp Micro kit from Qiagen did not gave rise to consistent results but the organic method coupled with an ultrafiltration step with the Microcon YM-100 concentrator (1) showed a promising STR profiling. For genotyping, we used a commercial kit from Applied BioSystems, the Identifiler, with 16 STR loci that include 13 CODIS (the FBI Laboratory's Combined DNA Index System) loci, one AMEL locus (amelogenin genes that are found on both the X and Y chromosomes; ref. 2), and two others (D2S1338 and D19S433). We succeeded in obtaining qualified STR profiles from 834 samples (Tables 3 and 4) and three examples are shown in Figure 1. At the TPOX locus (Figure 1, A and B), the allele 8 has an extremely high frequency and so do the 8 and 8/11 genotypes. TPOX shows the lowest discrimination power (DP), with the DP value of only 56.7%. The D3S1358 locus (Figure 1, C and D) has a more balanced allele frequency with a DP value of 74.35% but is lower than the FGA locus (Figure 1, E and F), which has a DP value of 84.97%. We also compared our data from the 13 CODIS loci to the references at the STRBase (www.cstl.nist.gov/div831/strbase) (Table 5).

Table 3 Genotypes and Their Frequencies in 15 Autosomal STR Loci

Locus	Genotype and Frequency									
CSF1PO	7/10	0.1318%	7/12	0.2635%	7/13	0.1318%	8/11	0.3953%	8/12	0.2635%
	8/13	0.1318%	9/10	1.0540%	9/11	2.2398%	9/12	1.5810%	9/13	0.2635%
	10	8.8274%	10/11	13.5705%	10/12	15.8103%	10/13	2.2398%	10/14	0.1318%
	11	10.6719%	11/12	20.4216%	11/13	2.6350%	11/14	0.1318%	12	13.3070%
	12/13	3.8208%	12/14	0.7905%	12/15	0.1318%	13	0.6588%	13/14	0.3953%
FGA	16	0.1263%	16/19	0.1263%	16/22	0.1263%	16/23	0.1263%	17	0.1263%
	17/21	0.2525%	17/26	0.1263%	18/20	0.5051%	18/21	0.7576%	18/22	0.2525%
	18/23	0.7576%	18/24	0.2525%	18/25	0.3788%	18/28	0.1263%	19	0.7576%
	19/20	0.8838%	19/21	2.1465%	19/21.2	0.2525%	19/22	2.9040%	19/22.2	0.3788%
	19/23	1.5152%	19/23.2	0.1263%	19/24	2.1465%	19/25	0.7576%	19/26	0.3788%
	19/27	0.2525%	20	0.8838%	20.2/21.2	0.1263%	20.2/22	0.1263%	20.2/23	0.2525%
	20/21	2.5253%	20/22	2.3990%	20/23	1.2626%	20/23.2	0.2525%	20/24	1.0101%
	20/24.2	0.1263%	20/25	0.8838%	20/25.2	0.1263%	20/26	0.5051%	20/27	0.2525%
	21	2.0202%	21.2/22	0.5051%	21.2/22.2	0.1263%	21.2/23	1.3889%	21.2/24	0.2525%
	21.2/24.2	0.2525%	21.2/25	0.1263%	21/21.2	0.6313%	21/22	6.0606%	21/22.2	0.2525%
	21/23	3.6616%	21/23.2	0.1263%	21/24	4.5455%	21/24.2	0.2525%	21/25	2.6515%
	21/26	1.2626%	21/27	0.2525%	21/28	0.3788%	22	5.3030%	22.2/23	0.3788%
	22.2/24	0.1263%	22.2/25	0.1263%	22.2/26	0.2525%	22/22.2	0.7576%	22/23	6.8182%
	22/23.2	0.2525%	22/24	4.2929%	22/24.2	0.5051%	22/25	3.2828%	22/25.2	0.3788%
	22/26	1.5152%	22/26.2	0.1263%	22/28	0.1263%	23	2.6515%	23.2	0.1263%
	23.2/24	0.5051%	23.2/25	0.1263%	23/23.2	0.3788%	23/24	4.6717%	23/24.2	0.6313%
	23/25	4.2929%	23/25.2	0.1263%	23/26	0.8838%	23/26.2	0.2525%	23/27	0.1263%
	23/28	0.1263%	24	1.7677%	24/24.2	0.1263%	24/25	2.6515%	24/26	0.6313%
	24/27	0.2525%	25	0.8838%	25.2/26	0.2525%	25.2/27	0.3788%	25/26	0.6313%
	25/27	0.5051%	25/28	0.1263%	26	0.3788%	26/27	0.2525%		
TH01	4/7	0.2475%	6	1.7327%	6/10	1.4851%	6/7	5.5693%	6/8	1.8564%
	6/9	9.0347%	6/9.3	2.5990%	7	6.5594%	7/10	3.3416%	7/8	4.4554%
	7/9	19.8020%	7/9.3	5.0743%	8	0.7426%	8.3/9	0.1238%	8.3/9.3	0.1238%
	8/10	1.2376%	8/9	5.8168%	8/9.3	2.1040%	9	13.8614%	9.3	1.8564%
	9.3/10	0.4950%	9/10	4.8267%	9/11	0.1238%	9/9.3	6.5594%	10	0.3713%
TPOX	6/11	0.3690%	8	32.9643%	8/10	3.1980%	8/11	29.8893%	8/12	1.9680%
	8/13	0.1230%	8/9	11.6851%	9	1.8450%	9/10	0.1230%	9/11	5.4121%
	9/12	0.3690%	10	0.1230%	10/11	2.5830%	10/12	0.1230%	11	8.3641%
	11/12	0.7380%	11/13	0.1230%						

<div align="center">**Table 3** *Continued*</div>

Locus	Genotype and Frequency									
vWA	13/19	0.2460%	14	4.1820%	14/15	1.9680%	14/16	6.5191%	14/17	12.4231%
	14/18	9.1021%	14/19	3.3210%	14/20	1.2300%	15	0.1230%	15/16	1.4760%
	15/17	2.9520%	15/18	0.7380%	15/19	1.2300%	15/20	0.1230%	16	1.8450%
	16/17	7.2571%	16/18	7.6261%	16/19	2.8290%	16/20	0.8610%	17	7.3801%
	17/18	9.7171%	17/19	6.5191%	17/20	0.8610%	17/21	0.2460%	17/22	0.1230%
	18	4.4280%	18/19	2.8290%	18/20	0.7380%	19	0.8610%	19/20	0.2460%
D3S1358	12/15	0.2466%	12/17	0.2466%	13/14	0.3699%	13/17	0.2466%	14	0.4932%
	14/15	3.6991%	14/16	3.6991%	14/17	2.2195%	14/18	0.8631%	15	6.9051%
	15/16	19.2355%	15/17	16.2762%	15/18	4.0691%	15/19	0.2466%	16	12.7004%
	16/17	13.6868%	16/18	4.3157%	16/19	0.3699%	16/20	0.2466%	17	4.8089%
	17/18	4.0691%	17/19	0.1233%	18	0.6165%	18/19	0.1233%	19	0.1233%
D5S818	7/10	0.9877%	7/11	1.1111%	7/12	0.8642%	7/13	0.2469%	8/10	0.1235%
	9	0.6173%	9/10	2.8395%	9/11	1.8519%	9/12	2.8395%	9/13	2.3457%
	10	5.6790%	10/11	9.6296%	10/12	10.8642%	10/13	8.6420%	10/14	0.7407%
	11	9.3827%	11/12	11.9753%	11/13	9.0123%	11/14	0.9877%	12	6.9136%
	12/13	8.1481%	12/14	0.2469%	12/15	0.2469%	13	3.3333%	13/14	0.2469%
	14	0.1235%								
D7S820	7/10	0.7926%	7/11	1.0568%	7/12	0.3963%	7/8	0.2642%	7/9	0.2642%
	8	4.6235%	8/10	6.4729%	8/11	12.2853%	8/12	6.6050%	8/13	1.0568%
	8/14	0.1321%	8/9	3.0383%	9	1.0568%	9/10	3.6988%	9/11	4.8877%
	9/12	1.8494%	9/13	0.7926%	9/14	0.1321%	10	2.7741%	10/11	10.1717%
	10/12	4.2272%	10/13	0.6605%	10/14	0.2642%	11	12.6816%	11/12	12.8137%
	11/13	1.7173%	11/14	0.1321%	12	3.6988%	12/13	1.3210%	13	0.1321%
D8S1179	8/12	0.4920%	8/14	0.1230%	8/15	0.2460%	9/13	0.2460%	9/14	0.1230%
	10	1.1070%	10/11	2.2140%	10/12	3.1980%	10/13	5.1661%	10/14	4.9200%
	10/15	6.1501%	10/16	2.8290%	10/17	0.6150%	11	0.9840%	11/12	1.4760%
	11/13	2.2140%	11/14	3.3210%	11/15	3.4440%	11/16	1.4760%	12	1.4760%
	12/13	5.5351%	12/14	4.0590%	12/15	4.4280%	12/16	1.5990%	12/17	0.1230%
	13	5.4121%	13/14	5.5351%	13/15	5.1661%	13/16	2.0910%	13/17	0.3690%
	14	3.6900%	14/15	7.3801%	14/16	3.3210%	14/17	0.8610%	14/18	0.2460%
	15	4.9200%	15/16	1.9680%	15/17	0.3690%	15/18	0.1230%	16	0.7380%
	16/17	0.2460%								
D13S317	6/8	0.1267%	7/11	0.2535%	7/9	0.1267%	8	9.2522%	8/10	4.4360%
	8/11	14.3219%	8/12	9.7592%	8/13	2.4081%	8/14	1.0139%	8/9	6.8441%
	9	1.5209%	9/10	3.8023%	9/11	5.4499%	9/12	4.3093%	9/13	1.1407%
	9/14	0.2535%	10	1.5209%	10/11	3.8023%	10/12	3.1686%	10/13	1.1407%
	10/14	0.3802%	11	4.9430%	11/12	9.1255%	11/13	3.0418%	11/14	0.2535%
	12	3.6755%	12/13	2.2814%	12/14	0.6337%	13	0.5070%	13/14	0.3802%
	14	0.1267%								
D16S539	8/11	0.7472%	8/12	0.4981%	8/9	0.7472%	9	5.9776%	9/10	2.8643%
	9/11	12.3288%	9/12	6.7248%	9/13	2.8643%	9/14	0.1245%	10	1.1208%
	10/11	6.8493%	10/12	5.4795%	10/13	2.7397%	10/14	0.4981%	11	9.3400%
	11/12	15.6912%	11/13	8.4682%	11/14	1.1208%	12	7.4720%	12/13	5.6040%
	12/14	1.1208%	12/15	0.1245%	12/16	0.1245%	13	1.1208%	13/14	0.2491%
D18S51	7/16	0.1300%	9/10	0.2601%	9/12	0.1300%	9/13	0.1300%	10/11	0.1300%
	10/13	0.2601%	10/14	0.1300%	10/15	0.1300%	11	0.3901%	11/13	0.2601%
	11/14	0.6502%	11/15	0.1300%	11/16	0.3901%	12	0.6502%	12/13	2.4707%
	12/14	1.6905%	12/15	2.6008%	12/16	1.5605%	12/17	0.7802%	12/18	0.5202%

Table 3 *Continued*

Locus	Genotype and Frequency									
D18S51	12/19	0.5202%	12/23	0.3901%	13	2.0806%	13.2	0.1300%	13.2/14	0.1300%
	13.2/15	0.1300%	13/14	4.6814%	13/15	6.5020%	13/16	4.2913%	13/17	2.7308%
	13/18	1.1704%	13/19	0.7802%	13/20	0.2601%	13/21	0.6502%	13/23	0.1300%
	13/25	0.1300%	14	3.6411%	14.2	0.1300%	14.2/15	0.2601%	14.2/16	0.1300%
	14.2/17	0.1300%	14/14.2	0.1300%	14/15	8.3225%	14/16	5.8518%	14/17	1.6905%
	14/18	1.8205%	14/19	1.6905%	14/20	0.7802%	14/21	0.7802%	14/22	0.9103%
	14/23	0.1300%	14/24	0.2601%	15	7.1521%	15/16	6.6320%	15/17	2.9909%
	15/18	2.0806%	15/19	0.7802%	15/20	0.2601%	15/21	0.6502%	15/22	0.1300%
	16	2.9909%	16/17	2.4707%	16/18	1.5605%	16/19	0.7802%	16/20	0.1300%
	16/21	0.3901%	16/22	0.6502%	16/24	0.2601%	17	1.4304%	17/18	0.7802%
	17/19	0.6502%	17/21	0.2601%	17/22	0.6502%	18	0.2601%	18/19	0.1300%
	18/21	0.1300%	18/23	0.1300%	18/25	0.1300%	19/21	0.3901%	20	0.1300%
	20/21	0.1300%	22	0.1300%						
D21S11	27/28	0.2484%	27/29	0.2484%	27/31.2	0.2484%	27/32.2	0.1242%	27/34.2	0.1242%
	28	0.9938%	28.2/30	0.1242%	28.2/31.2	0.2484%	28/29	4.5963%	28/30	2.7329%
	28/30.2	0.7453%	28/31	1.1180%	28/31.2	1.1180%	28/32	0.3727%	28/32.2	2.4845%
	28/33.2	0.7453%	29	7.2050%	29.2/30	0.4969%	29/29.2	0.1242%	29/30	12.1739%
	29/30.2	1.4907%	29/31	2.6087%	29/31.2	3.8509%	29/32	0.9938%	29/32.2	5.9627%
	29/33.2	2.2360%	29/34.2	0.3727%	29/35.2	0.2484%	30	6.8323%	30.2/31	0.4969%
	30.2/31.2	0.3727%	30.2/32	0.2484%	30.2/32.2	0.4969%	30.2/33.2	0.8696%	30/30.2	1.7391%
	30/31	3.7267%	30/31.2	2.9814%	30/32	0.9938%	30/32.2	7.5776%	30/33.2	3.2298%
	30/34.2	0.3727%	31	0.6211%	31.2	1.1180%	31.2/32	0.4969%	31.2/32.2	2.4845%
	31.2/33.2	0.6211%	31.2/34.2	0.1242%	31/31.2	1.2422%	31/32	0.2484%	31/32.2	1.6149%
	31/33	0.1242%	31/33.2	1.1180%	32.2	2.3602%	32.2/33	0.2484%	32.2/33.2	1.4907%
	32.2/34.2	0.3727%	32/32.2	0.7453%	32/33.2	0.7453%	33.2	0.2484%		
D2S1338	16	0.1297%	16/17	0.3891%	16/18	0.1297%	16/19	0.5188%	16/20	0.2594%
	16/22	0.2594%	16/23	0.6485%	16/24	0.2594%	16/25	0.2594%	17	2.4643%
	17/18	1.5564%	17/19	5.4475%	17/20	2.3346%	17/21	0.7782%	17/22	1.2970%
	17/23	3.7613%	17/24	3.3722%	17/25	1.5564%	17/26	0.3891%	18	2.4643%
	18/19	4.2802%	18/20	2.0752%	18/21	0.2594%	18/22	1.0376%	18/23	2.8534%
	18/24	2.3346%	18/25	0.6485%	19	4.1505%	19/20	4.1505%	19/21	1.2970%
	19/22	1.6861%	19/23	6.0960%	19/24	3.6316%	19/25	1.9455%	20	2.3346%
	20/21	0.6485%	20/22	1.0376%	20/23	3.1128%	20/24	2.3346%	20/25	1.5564%
	20/26	0.3891%	21	0.5188%	21/22	0.3891%	21/23	1.9455%	21/24	0.9079%
	21/25	0.6485%	22	1.1673%	22/23	1.6861%	22/24	1.2970%	22/25	0.5188%
	22/26	0.1297%	23	3.2425%	23/24	4.2802%	23/25	2.2049%	23/26	0.2594%
	24	2.2049%	24/25	1.8158%	24/26	0.2594%	25	0.2594%	25/26	0.1297%
D19S433	9/13	0.2466%	9/14	0.6165%	9/14.2	0.3699%	9/15.2	0.6165%	9/16	0.2466%
	11.2/14	0.4932%	11/14.2	0.1233%	12	0.3699%	12.2/14	0.3699%	12.2/14.2	0.1233%
	12.2/15	0.2466%	12.2/15.2	0.2466%	12/13	3.0826%	12/13.2	0.2466%	12/14	2.9593%
	12/14.2	0.8631%	12/15	0.6165%	12/15.2	1.4797%	12/16	0.2466%	12/16.2	0.2466%
	13	5.6720%	13.2	0.2466%	13.2/14	2.5894%	13.2/14.2	0.4932%	13.2/15	0.4932%
	13.2/15.2	1.1097%	13.2/16	0.1233%	13.2/16.2	0.2466%	13/13.2	3.3292%	13/14	14.7965%
	13/14.2	5.1788%	13/15	4.0691%	13/15.2	6.4118%	13/16	0.9864%	13/16.2	0.9864%
	13/17.2	0.2466%	13/18.2	0.2466%	14	6.0419%	14.2	0.9864%	14.2/15	1.2330%
	14.2/15.2	2.3428%	14.2/16.2	0.9864%	14/14.2	3.8224%	14/15	4.0691%	14/15.2	8.3847%
	14/16	1.1097%	14/16.2	1.2330%	15	1.7263%	15.2	2.3428%	15.2/16.2	0.6165%
	15.2/17	0.1233%	15/15.2	2.5894%	15/16	0.2466%	15/16.2	0.3699%	15/17.2	0.2466%
	16	0.2466%	16.2	0.1233%	16/16.2	0.1233%				

Table 4 Statistics of 15 Identifiler Loci Common Index

Locus	Allele No.	Genotype No.	PIC	PICe	Pm	Pme	DP	DPe	EP
CSF1PO	9	25	66.53%	66.47%	33.47%	28.14%	66.53%	71.86%	49.56%
FGA	20	99	84.97%	86.10%	15.02%	12.63%	84.97%	87.37%	75.73%
TH01	9	25	74.88%	72.67%	25.12%	23.95%	74.88%	76.05%	57.23%
TPOX	7	17	56.70%	53.18%	8.39%	6.76%	56.70%	59.14%	36.15%
vWA	10	30	81.18%	77.73%	18.82%	19.47%	81.18%	80.53%	63.42%
D3S1358	9	25	74.35%	69.79%	25.65%	25.79%	74.35%	74.21%	53.31%
D5S818	9	26	73.95%	74.83%	26.05%	21.69%	73.95%	78.31%	59.44%
D7S820	8	30	75.03%	75.03%	24.97%	21.87%	75.03%	78.13%	60.00%
D8S1179	11	41	81.67%	82.85%	18.33%	15.27%	81.67%	84.73%	70.60%
D13S317	9	31	78.45%	77.38%	21.55%	19.80%	78.45%	80.19%	63.01%
D16S539	9	25	74.97%	74.18%	25.03%	22.40%	74.97%	77.60%	58.79%
D18S51	20	82	80.88%	83.88%	19.12%	14.51%	80.88%	85.49%	72.37%
D21S11	15	59	80.62%	81.52%	19.38%	16.55%	80.62%	83.45%	69.04%
D2S1338	11	60	81.06%	86.04%	18.94%	12.64%	81.06%	87.36%	75.49%
D19S433	16	58	82.24%	80.30%	17.76%	17.56%	82.24%	82.43%	67.33%
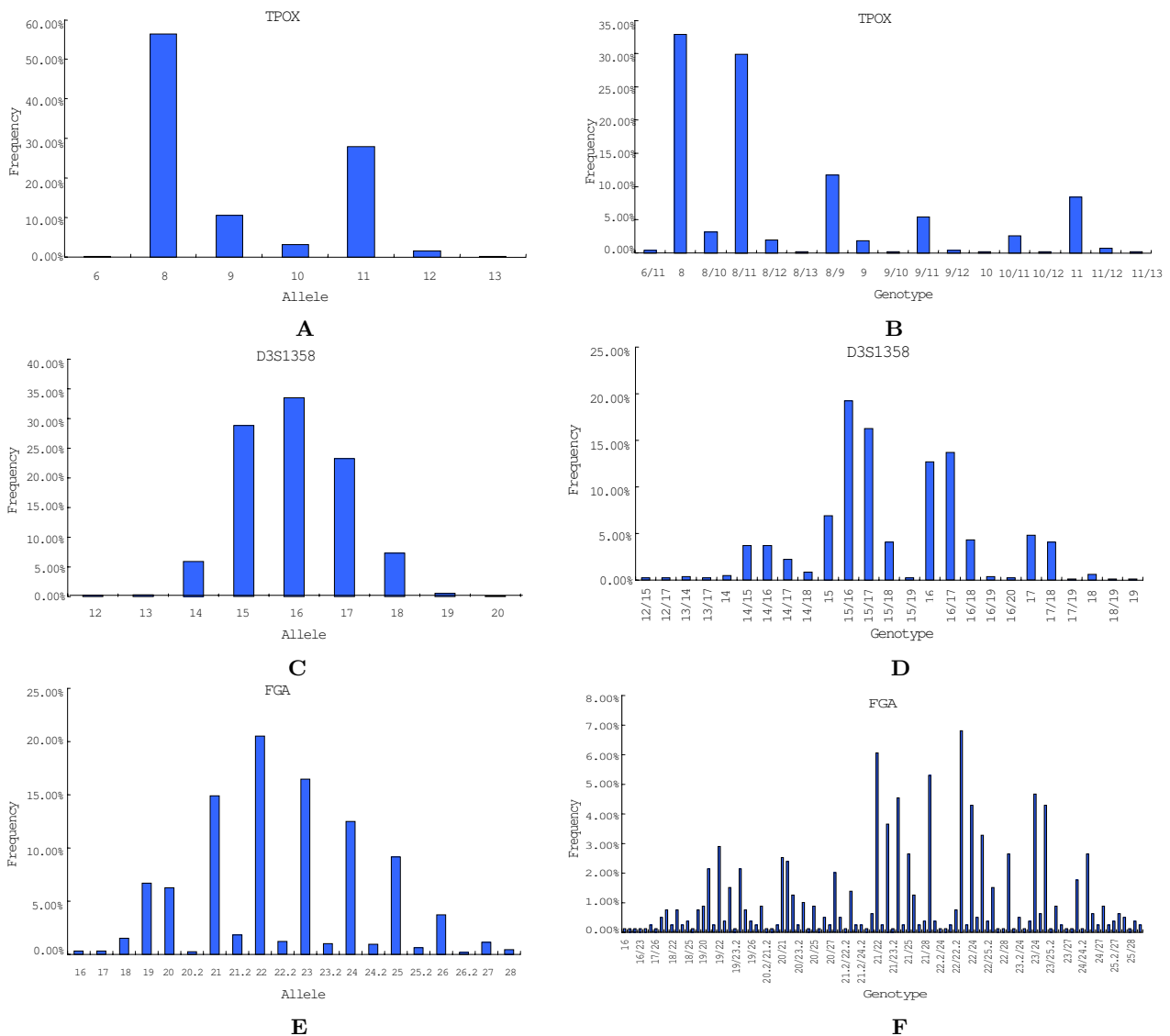
Fig. 1 Allele and genotype frequencies from three representative loci: TPOX (A and B), D3S1358 (C and D), and FGA (E and F).

Table 5 Comparison of Allele Numbers from 13 CODIS Loci Between the Thai Tsunami
Victims and the STR Base Reported Population

Locus	Detected allele	Reported allele	Detected/Reported
CSF1PO	9	12	75.0%
FGA	20	67	29.9%
TH01	9	20	45.0%
TPOX	7	10	70.0%
vWA	10	26	38.5%
D3S1358	9	20	45.0%
D5S818	9	10	90.0%
D7S820	8	22	36.4%
D8S1179	11	13	84.6%
D13S317	9	14	64.3%
D16S539	9	10	90.0%
D18S51	20	42	47.6%
D21S11	15	72	20.8%

Discussion

Nuclear DNA is degraded in tooth specimens but mt-DNA from them is recoverable

We had quite a struggle in extracting enough DNA for nuclear markers from tooth specimens initially due to the variable amount of tooth samples (decomposed bodies often have missing teeth to different extents). Despite various controls, careful planning for the experiments, and DNA enrichment procedures, the amount of DNA purified from an entire tooth or pooled from multiple extraction procedures was not enough to give rise to satisfactory results for all nuclear loci; some of the samples did not show any evidence of remaining DNA. When some of the specimens did give positive results, the quality was often poor and unusable. Having worked at the sites of specimen collection, we observed that the victim bodies stored in the local morgue were not maintained in low temperature and most of them had been washed in badly contaminated sea water for days before sample retrieval. It was reported by numerous investigators that DNA degrades rather quickly when exposed to high temperature (3); salt and bacterial contaminations are also inhibitory factors for appropriate DNA preparation and amplification (4, 5). Furthermore, the amount of recoverable DNA is also critical since low DNA concentration often causes false positive results. Finally, the procedure to collect teeth, though done by forensic professionals, is different from anthropologists' procedures in which the teeth are often collected with the skull. When teeth are removed, the cavity is exposed to air and also becomes accessible to microbes. As a result, we encountered tremendous difficulty in recovering DNA in an adequate amount (often lower than 3 pg in total pooled extracts) to perform our experiments that insist information as complete as possible.

Since these specimens are very precious and important for the victims' family to identify their beloved ones lost in the tragedy, we attempted to rescue mt-DNA in case of request. We chose to genotype loci in the non-coding region of mitochondrial sequences, the displacement loop (D-loop, sometimes referred as the control region) that is approximately 1,100 bp in length. The forensic value of mt-DNA relies on the sequence variability of the D-loop between individuals, and the maternally inherited nature of mitochondria makes it suitable for matching blood relatives of maternal inheritance. In addition to skin, blood, semen, saliva, and the usual body remains for forensic DNA identification, mt-DNA has also been extracted from teeth and used for such purpose (6, 7). Because the tooth specimens of the tsunami victims were badly degraded and could not be used to obtain satisfactory nuclear STR profiling, we decided to sequence mt-DNA from the tooth specimens to salvage as much information as we can. The complete sequences from the multiple hypervariable regions often enable investigators to identify remains of war casu-

alties and individuals involved in mass disasters or criminal case (8). We indeed achieved a 51% success rate in recovering mt-DNA information, ready to serve any relatives who step up for DNA matching in searching for missing family members in the disaster.

Bones are better specimens for typing nuclear marks but an enrichment step is of essence

Realizing that similar situation may happen in preparing DNA from the bones, we designed a series of experiments to work out a standard protocol for tsunami victims. We first investigated commercial kits from companies whose DNA purification kits are widely used in forensics. The IQ$^{\text{TM}}$ system from Promega and the QIAamp Micro kit from Qiagen are two examples for DNA extraction, which are successfully used to extract DNA from blood stains, buccal swabs, hair follicles, sperms, teeth, and bones. We also tested in parallel several classical methods. Among them, a classical organic method used to prepare DNA from calcified tissues (4), coupled with an sample concentration step with the Microcon YM-100 concentrator, an ultrafiltration unit, gave us the most satisfactory result. Among those complicated reasons, the most decisive factors are the relative purity of the resulted DNA preparations and the higher yield

of the procedure. The yields of this protocol is often ten times higher than the commercial kits when start with the same amount raw bone samples. Another important notion from our experience is that the bone specimen is not limited by size, and can be readily grinded into a fine powder for DNA extraction, yielding a higher concentration of DNA with minimal degradation. Relatively pure and high concentration of the DNA samples gave us consistent results for the nuclear STR typing.

The Thai tsunami victims are ethnic diverse group based on STR profiles

The Thai tsunami victims are an admixture of foreign tourists and local residents, including not only Thai nationals, but also other Asians, Europeans, Americans, and so on. As we anticipated, the results show a great diversity among the typed loci from the victims when compared to any of the reference population-based data. Comparing the values of the observed heterozygosity (Ho) and expected heterozygosity (He) to those of U.S. Caucasian, African American, Hispanic, Chamorro, or Filipino populations, we found that the Thai tsunami victims generally have lower Ho and He values than those of the U.S. Caucasian and African American populations (Tables 6 and 7).

Table 6 Comparison of Allele Frequencies from 15 STR Loci Between the Thai Tsunami Victims and Other Reference Populations

Locus	Allele	Thai tsunami victim (N=834)	U.S. Caucasian (N=302)	African American (N=258)	Locus	Allele	Thai tsunami victim (N=834)	U.S. Caucasian (N=302)	African American (N=258)
CSF1PO	7	0.003	–	0.053	FGA	19	0.067	0.053	0.062
	8	0.004	0.005	0.060		19.2	–	–	0.004
	9	0.026	0.012	0.037		20	0.063	0.127	0.056
	9.3	–	–	0.257		20.2	0.003	–	–
	10	0.253	0.217	–		21	0.149	0.185	0.116
	11	0.304	0.301	0.249		21.2	0.018	0.005	–
	12	0.348	0.361	0.298		22	0.205	0.219	0.196
	13	0.055	0.096	0.037		22.2	0.012	0.012	0.004
	14	0.007	0.008	0.010		22.3	–	–	0.002
	15	0.001	–	–		23	0.165	0.134	0.171
	Ho	0.665	0.725	0.759		23.2	0.010	0.003	0.002
	He	0.667	0.724	0.776		24	0.125	0.136	0.122
FGA	16	0.003	–	–		24.2	0.009	0.002	–
	16.2	–	–	0.022		25	0.092	0.071	0.124
	17	0.003	–	–		25.2	0.006	–	–
	18	0.015	0.026	0.002		26	0.037	0.023	0.081
	18.2	–	–	0.012		26.2	0.002	–	–

Table 6 *Continued*

Locus	Allele	Thai tsunami victim (N=834)	U.S. Caucasian (N=302)	African American (N=258)	Locus	Allele	Thai tsunami victim (N=834)	U.S. Caucasian (N=302)	African American (N=258)
FGA	27	0.011	0.003	0.023	D3S1358	15	0.288	0.262	0.302
	28	0.004	–	0.012		15.2	–	–	0.002
	29	–	–	0.004		16	0.335	0.253	0.335
	30	–	–	0.002		17	0.232	0.215	0.205
	30.2	–	–	0.002		18	0.073	0.152	0.060
	31.2	–	–	0.002		19	0.006	0.012	0.004
	Ho	0.850	0.887	0.884		20	0.001	0.002	–
	He	0.861	0.857	0.876		Ho	0.744	0.765	0.764
TH01	4	0.001	–	–		He	0.698	0.789	0.744
	5	–	0.002	0.004	D5S818	7	0.016	0.002	–
	6	0.120	0.232	0.124		8	0.001	0.003	0.048
	7	0.258	0.190	0.421		9	0.056	0.050	0.039
	8	0.085	0.184	0.194		10	0.226	0.051	0.070
	8.3	0.001	–	–		11	0.267	0.361	0.233
	9	0.370	0.114	0.151		12	0.245	0.384	0.353
	9.3	0.103	0.368	0.105		13	0.177	0.141	0.238
	10	0.061	0.008	0.006		14	0.012	0.007	0.016
	11	0.001	0.002	–		15	0.001	0.002	0.004
	Ho	0.749	0.719	0.760		Ho	0.740	0.709	0.733
	He	0.727	0.756	0.738		He	0.740	0.698	0.757
TPOX	5	–	0.002	–	D7S820	6	–	–	0.002
	6	0.002	0.002	0.101		7	0.014	0.018	0.016
	7	–	–	0.017		8	0.196	0.151	0.236
	8	0.564	0.535	0.372		8.1	–	0.002	–
	9	0.106	0.119	0.178		9	0.084	0.177	0.109
	10	0.031	0.056	0.089		9.3	–	–	0.002
	11	0.279	0.243	0.219		10	0.159	0.243	0.331
	12	0.016	0.041	0.021		11	0.342	0.207	0.203
	13	0.001	0.002	0.002		12	0.173	0.166	0.087
	Ho	0.567	0.656	0.764		13	0.029	0.035	0.014
	He	0.532	0.637	0.764		14	0.003	0.002	–
vWA	12	–	–	0.002		Ho	0.750	0.818	0.764
	13	0.001	0.002	0.008		He	0.750	0.816	0.775
	14	0.215	0.094	0.078	D8S1179	8	0.004	0.012	0.002
	15	0.044	0.111	0.186		9	0.002	0.003	0.006
	16	0.151	0.200	0.248		10	0.137	0.101	0.029
	17	0.274	0.281	0.242		11	0.081	0.083	0.045
	18	0.198	0.200	0.155		12	0.119	0.185	0.141
	19	0.095	0.104	0.062		13	0.186	0.305	0.217
	20	0.020	0.005	0.016		14	0.186	0.166	0.300
	21	0.001	0.002	0.004		15	0.196	0.114	0.184
	22	0.001	–	–		16	0.075	0.031	0.070
	Ho	0.812	0.841	0.802		17	0.013	–	0.004
	He	0.777	0.810	0.813		18	0.002	–	0.002
D3S1358	11	–	0.002	–		Ho	0.817	0.778	0.764
	12	0.002	–	–		He	0.829	0.816	0.803
	13	0.003	–	0.002	D13S317	6	0.001	–	–
	14	0.059	0.103	0.089		7	0.002	–	–

Table 6 *Continued*

Locus	Allele	Thai tsunami victim (N=834)	U.S. Caucasian (N=302)	African American (N=258)	Locus	Allele	Thai tsunami victim (N=834)	U.S. Caucasian (N=302)	African American (N=258)
D13S317	8	0.287	0.113	0.033	D21S11	28	0.081	0.159	0.258
	9	0.125	0.075	0.033		28.2	0.002	–	–
	10	0.099	0.051	0.023		29	0.247	0.195	0.198
	11	0.231	0.339	0.306		29.2	0.003	0.003	–
	12	0.183	0.248	0.424		30	0.249	0.278	0.174
	13	0.057	0.124	0.145		30.2	0.032	0.028	0.010
	14	0.016	0.048	0.035		31	0.068	0.083	0.081
	15	–	0.002	–		31.2	0.080	0.099	0.047
	Ho	0.785	0.745	0.690		32	0.024	0.007	0.008
	He	0.774	0.786	0.702		32.2	0.142	0.084	0.058
D16S539	8	0.010	0.018	0.039		33	0.002	0.002	0.006
	9	0.188	0.113	0.196		33.1	–	–	0.002
	10	0.103	0.056	0.116		33.2	0.058	0.026	0.035
	11	0.319	0.321	0.318		34	–	–	0.006
	12	0.252	0.326	0.196		34.2	0.007	0.005	–
	13	0.111	0.146	0.118		35	–	0.002	0.023
	14	0.016	0.020	0.017		35.2	0.001	–	–
	15	0.001	–	–		36	–	–	0.010
	16	0.001	–	–		37	–	–	0.002
	Ho	0.750	0.735	0.783		38	–	–	0.002
	He	0.742	0.754	0.795		39	–	–	0.002
D18S51	7	0.001	–	–		Ho	0.806	0.841	0.830
	9	0.003	–	0.004		He	0.815	0.835	0.845
	10	0.005	0.008	0.006	D2S1338	15	–	0.002	–
	11	0.012	0.017	0.002		16	0.015	0.033	0.058
	12	0.060	0.127	0.078		17	0.129	0.182	0.099
	13	0.143	0.132	0.053		18	0.101	0.079	0.039
	13.2	0.003	–	0.006		19	0.187	0.114	0.148
	14	0.185	0.137	0.072		20	0.113	0.146	0.103
	14.2	0.005	0.002	–		21	0.040	0.041	0.144
	15	0.230	0.159	0.161		22	0.058	0.038	0.130
	15.2	–	–	0.002		23	0.167	0.118	0.111
	16	0.156	0.139	0.158		24	0.125	0.123	0.080
	17	0.080	0.126	0.152		25	0.059	0.093	0.072
	18	0.045	0.076	0.123		26	0.008	0.030	0.012
	19	0.029	0.038	0.099		27	–	0.002	0.004
	20	0.009	0.022	0.064		Ho	0.811	0.871	0.903
	21	0.017	0.008	0.010		He	0.860	0.885	0.893
	21.2	–	–	0.002	D19S433	9	0.010	–	–
	22	0.013	0.008	0.006		10	–	0.002	0.010
	23	0.004	–	0.002		11	0.001	0.005	0.062
	24	0.003	–	0.002		11.2	0.002	–	–
	25	0.001	–	–		12	0.052	0.081	0.114
	Ho	0.809	0.881	0.860		12.2	0.005	0.002	0.035
	He	0.839	0.880	0.885		13	0.255	0.253	0.246
D21S11	25.2	–	0.002	–		13.2	0.046	0.007	0.052
	26	–	–	0.002		14	0.263	0.369	0.223
	27	0.005	0.026	0.078		14.2	0.088	0.018	0.079

<div align="center">**Table 6** *Continued*</div>

Locus	Allele	Thai tsunami victim (N=834)	U.S. Caucasian (N=302)	African American (N=258)	Locus	Allele	Thai tsunami victim (N=834)	U.S. Caucasian (N=302)	African American (N=258)
D19S433	15	0.088	0.152	0.078	D19S433	17.2	0.002	0.002	0.006
	15.2	0.143	0.035	0.060		18.2	0.001	0.002	0.004
	16	0.018	0.050	0.004		Ho	0.822	0.755	0.876
	16.2	0.025	0.015	0.027		He	0.803	0.767	0.854
	17	0.001	0.008	–					

<div align="center">**Table 7 Comparison of Allele Frequencies from Two STR Loci Between the Thai Tsunami Victims and Other Five Reference Populations**</div>

Locus	Allele	Thai tsunami victim (N=834)	African American (N=167)	U.S. Caucasian (N=152)	Hispanic (N=142)	Chamorro (N=72)	Filipino (N=71)
D2S1338	16	0.0149	0.0449	0.0296	0.0176	0.0278	0.0282
	17	0.1291	0.1018	0.1941	0.2218	0.1042	0.0775
	18	0.1005	0.0659	0.0526	0.0423	0.0833	0.0563
	19	0.1868	0.1377	0.1447	0.2601	0.1875	0.2183
	20	0.1128	0.0630	0.1546	0.1409	0.1111	0.0775
	21	0.0396	0.1527	0.1974	0.0106	0.0139	0.0423
	22	0.0584	0.1377	0.0296	0.0704	0.0972	0.0634
	23	0.1667	0.9880	0.1349	0.1232	0.1736	0.1338
	24	0.1245	0.0928	0.1217	0.0669	0.1319	0.2606
	25	0.0590	0.0838	0.0954	0.0387	0.0556	0.0352
	26	0.0078	0.0210	0.0230	0.0070	0.0069	0.0070
	27	–	–	–	–	0.0069	–
D19S433	9	0.0105	–	–	0.0035	–	–
	10	–	0.0150	–	–	–	–
	11	0.0006	0.0689	–	0.0035	–	–
	11.2	0.0025	–	–	–	–	–
	12	0.0524	0.1138	0.1086	0.0563	0.0347	0.0282
	12.2	0.0049	0.0808	0.0066	0.0211	0.0139	–
	13	0.2546	0.2964	0.2828	0.1620	0.3542	0.2887
	13.2	0.0456	0.0509	0.0263	0.1092	0.0417	0.0423
	14	0.2626	0.1976	0.3355	0.3204	0.2292	0.1549
	14.2	0.0875	0.0539	0.0033	0.0458	0.0972	0.0493
	15	0.0881	0.0389	0.1349	0.1197	0.0903	0.1056
	15.2	0.1430	0.0389	0.0263	0.0810	0.0972	0.2465
	16	0.0179	0.0210	0.0428	0.0423	–	0.0141
	16.2	0.0253	0.0180	0.0263	0.0352	0.0139	0.0634
	17	0.0006	–	–	–	–	–
	17.2	0.0024	0.0030	0.0033	–	0.0278	0.0070
	18.2	0.0012	0.0030	0.0033	–	–	–

Conclusion

As the final note to this report, we are happy to announce the completion of the project after handing all our data to the Thai Tsunami Victims Identification Center (TTVI center), since we are only responsible for the postmortem DNA profiling and the victim identification process. We have learnt recently that

over 200 relatives of the tsunami victims have already found the bodies of their family members, who are indeed identified by the DNA method, including both Thai nationals and foreign tourists from several nations.

Materials and Methods

Sample collection and processing

Bone and tooth specimens were collected by certified forensic scientists and shipped on dry ice to our forensic laboratory. After data entry, each sample was decontaminated and processed according to standard forensic DNA extraction protocols. Tooth marrow tissues were collected and DNA was extracted with commercial kits (DNA IQ^{TM} System, Promega Corp., Madison, USA; QIAamp DNA Micro Kit, Qiagen, Inc., Hilden, Germany).

The bone specimen of approximately 2 g was cut into 5×5×5 mm pieces and washed in 50-mL Falcon tubes with Terg-A-Zyme (an enzyme-active powdered detergent made by Alconox, Inc., New York, USA) just enough to cover the samples. The mixture was sonicated for 30 min, rinsed thoroughly with distilled water, and dried at 56°C in an incubator for more than 2 h. The dried bone pieces were milled in a SPEX CertiPrep 6750 freezer mill (SPEX CertiPrep, Inc., Metuchen, USA). DNA samples extracted from the fine bone powders were concentrated with Microcon YM-100 concentrators (Millipore Corp., Billerica, USA) (*1*).

DNA amplification and genotyping

For STR analysis, the AmpFLSTR$^{®}$ Identifiler$^{®}$ PCR amplification kit (Applied Biosystems, Foster City, USA) was used for PCR amplification and the results were analyzed with the 3730XL DNA Analyzer (Applied Biosystems) and the Genemapper software. For mt-DNA amplification, two pairs of primers specific to the human mt-DNA in the hyper-variable regions were used (HV1 primers: 1F/ctcacgggagctctccatgc and 1R/ggcggtatgcactttt-aacag; HV2 primers: 2F/ccaccattagcacccaaagc and 2R/tcccttgaccaccatcctc). The mitochondiral data were analyzed with the DNAStar package.

DNA was amplified in a total volume of 25 μL, containing 4.5 μL template DNA, 2.5 μL primers (1 pmol/μL), 2.5 μL dNTP (2.5 mM), 2 μL Mg^{2+}(25 mM), 2.5 μL 10× LA Buffer, 1 μL Taq polymerase (5 U/μL), and 10 μL ddH$_2$O. The PCR cycles were set as: denaturation at 95°C for 2 min, amplification at 95°C/60°C/72°C for 30 s respectively in a total of 35 cycles, and final extension at 72°C for 10 min. The PCR products were purified with a Millipore 96-well purification plate. Mitochondrial samples were sequenced on the 3730XL DNA Analyzer with Dye Terminator kits (GE Healthcare, USA).

References

1. Bulter, J.M. 2005. *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers* (second edition). Academic Press, New York, USA.

2. Iwase, M., *et al.* 2003. The amelogenin loci span an ancient pseudoautosomal boundary in diverse mammalian species. *Proc. Natl. Acad. Sci. USA* 100: 5258-5263.

3. Iwamura, E.S., *et al.* 2004. Human identification and analysis of DNA in bones. *Rev. Hosp. Clin. Fac. Med.* (Sao Paulo) 59: 383-388.

4. Alonso, A., *et al.* 2001. DNA typing from skeletal remains: evaluation of multiplex and megaplex STR systems on DNA isolated from bone and teeth samples. *Croat. Med. J.* 42: 260-266.

5. Burgi, S.B. (ed.) 1997. *First European-American intensive course in PCR based clinical and forensic testing.* Laboratory manual. Split, Croatia.

6. Primorac, D., *et al.* 1996. Identification of war victims from mass graves in Croatia, Bosnia, and Herzegovina by use of standard forensic methods and DNA typing. *J. Forensic Sci.* 41: 891-894.

7. Butler, J.M., *et al.* 2003. Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J. Forensic Sci.* 48: 908-911.

8. Budowle, B., *et al.* 2001. Population data on the STR loci D2S1338 and D19S433. *Forensic Sci. Commu.* 3: 3.