AUTOMATED FLUORESCENT SEQUENCING OF MITOCHONDRIAL DNA FOR ITALIAN POPULATION FREQUENCY DATA.

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Introduction

The analysis of mitochondrial DNA (mt DNA) sequence is a method to establish mtDNA type. In the context of forensic science a determination of the frequency of the different mtDNA types in the population must be done.

In this study we present population databases from 100 unrelated individuals representative of all italian regions. The mtDNA regions subjected to analysis was HVI (16.024-16365) and HVII (73-340). In both HVI and HVII, two sets of primers was used to amplify the regions of the mtDNA control region.

Mitochondrial DNA sequencing was performed with fluorescence-basd automated sequencer (373 A, ABD of P.E.,CA) from PCR products purified by filtration in a Microcon-100. Two different types of chemistries was employed.

A calculation of the frequency of a mtDNA type is the number of times the specific sequence has been observed, divided by the number of samples in the databases. This counting method was adopted only with DNA sequence information that was free from ambiguities within the region of comparison.

Material and Methods

DNA Preparation
Whole Blood
Genomic DNA, extracted from 500 µl of whole blood with phenol/clorophorm procedure, was quantified by spectrophotometry.

DNA Amplification
Mitochondrial DNA

Oligonucleotides primer specific for HV1 (16,024-16,365) and HV2 (73-340) regions were prepared.Two chimeric primers combining the M13-21 primer sequence with the mt-specific sequences and a primer with biotin at the 5' end were also synthesized.
All PCRs were performed in two-stage amplification. In the first-stage primers A and B were used to amplify a 1333-bp segment of human mtDNA of the hypervariable region 1 (HV1). Primers C and B were used to amplify a 1200-bp segment of the hypervariable region 2 (HV2). The PCR conditions were: in a total volume of 25 µl were mixed 50 ng whole blood DNA or 5 µl of nonquantitated bone DNA as template, primers at 1µM concentration. Amplification products were analyzed on 5% horizontal polyacrylamide gel stained with silver. In the second stage primers C and F were used to amplify a 400-bp segment of the hypervariable region 1 (HV1). Primers P1 and P2 were used to amplify a 395-bp segment of the hypervariable region 2 (HV2) at 0.5 µM final concentration. The primers F and P2 were chimeric.

REGION HV1 (16024-16365)		REGION HV2 (20-340)	
PRIMERS	POSITION	PRIMERS	POSITION
A	L15926	B	H00580
B	H00580	C	L15997
C	L15997	P1	L20
F	H16401	P2	H00395

DNA Sequencing

6µl of bone and 4 µl whole blood of the PCR solution with and without purification in Centricon 100 (Amicon Corp.) were used for sequencing reactions. The sequencing was carried out according to the DyeDeoxy™ -Terminator and Dye-Primer Cycle Sequencing Kit (ABD,PE). The reactions were denatured for 4 min at 95° C and loaded onto a 6% acrylamide gel. Electrophoresis and sequencing analysis were performed with an Applied Biosystems Mod. 373A DNA sequencer. Sequence comparisons were made using ABD SeqEd (sequence editing) software.(FIG. 1)

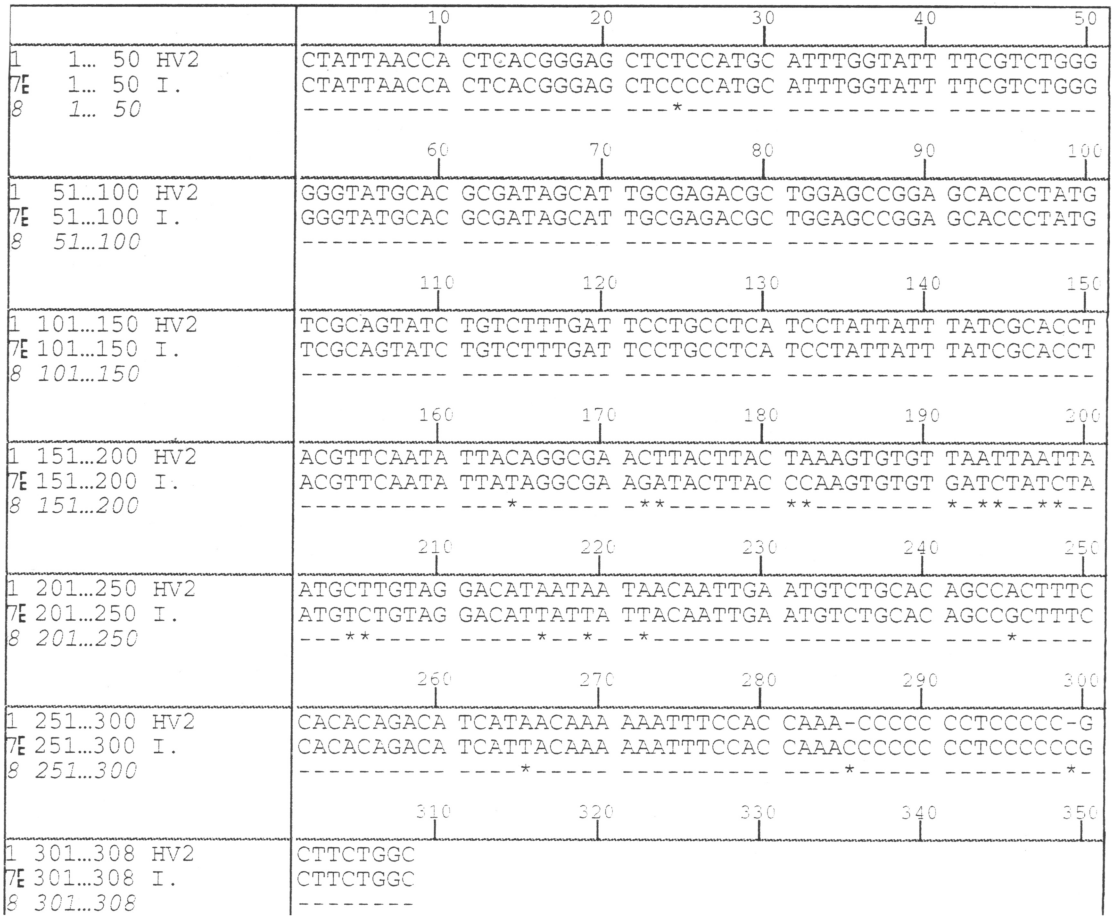

Figure 1 . Sequence information generated by directly comparison with reference sequence (ANDERSON) using Seq ED Software.

Results

Several strategies are currently available for the analysis of a DNA profile. Last tecnique used in forensic caseworks involves the direct sequencing of amplification product of the control region of mitochondrial DNA (HVR1 - HVR2). This region is highly polymorphic and presents different types of single nucleotide substitutions. In this case a single base substitution represents a different mtDNA type. In order to defined the different mtDNA type in Italian population, the hypervariable Region 1 and hypervariable Region 2 have been sequenced.
The region HV1 is between 16,000 to 16,400, and HV2 is located on the other side, approximately 10 to 320.
Therefore, a list of the mtDNA types of the individuals in Italian population has been done with respect to Anderson sequence. A calculation of the frequency of a mtDNA type is the number of times the specific sequence has been observed, divided by the number of samples in the databases. This counting method was adopted only with DNA sequence information that was free from ambiguities within the region of comparison.

This database include a sample of about 100 individuals collected from different regions of Italy. An example of some data from our database is show in figure 2.
We have found no deviation from Anderson sequence in only one sample. A statistical estimate of the results showed that 100 individuals defined 100 different mtDNA type. Therefore the frequency of a mtDNA type is about 0.01. This frequency might be multipled by allele frequencies of polymorphic nuclear loci to increase the power discrimination of the DNA test.

Reference

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Sullivan K.M., Hopgood R., Lang B. Gill P. "Automated amplification and sequencing of human mitochondrial DNA."1991.Electrophoresis 12:17-21

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Region HV1

Sequences	16094	16108	16142	16145	16152	16158	16162	16176	16177	16179	16189	16198	16205	16209	16214	16216	16218	16223	16225	16245	16256	16280	16282	16298	16325	16332	16348	16382	16390	16401
Anderson	T	C	C	G	T	A	A	C	A	C	T	T	C	T	C	A	C	C	C	C	C	A	C	T	T	C	C	C	G	C
Sample 1	C			A			T	G	G	T				A				T		G		C	G				T	T	A	
Sample 2						T						A													A					
Sample 3														A					G							G				
Sample 4														A			G									G				
Sample 5			G		A									A																
Sample 6																	G					C				G				G
Sample 7										T				A																
Sample 8		G					T			T						G			G			C		C						
Sample 9											C		G							G			A							
Sample 10											C		G		G						G		G							

Region HV2

Sequences	10	14	21	25	30	32	42	48	49	55	57	60	73	75	77	79	85	90	97	110	122	127	131	146	152	164	172	173	181	182	188	191	192	195	200	201	210	211	215	222	223	234	237	241	256	263	271	285	286	291	306,1	315,1
Anderson	T	T	A	A	T	A	T	C	A	T	T	T	A	G	A	G	G	G	G	C	C	T	T	T	T	C	T	T	A	C	A	A	T	T	T	A	T	A	A	C	T	A	A	A	C	A	C	C	A	A	¦	¦
Sample 1					C								T																	T			G		C	C	G	T	T	T	C	T	T	T		G			T		+ C	+ C
Sample 2																																	G														G						+ C
Sample 3			C				C					C	G									C	C			G	G		T																G	A					+ C
Sample 4		C																					C		C	G			T		G														G	G	A				
Sample 5													G			C				A			C											A													G					+ C
Sample 6	G						C					C	G														G				G	G		C												G		T				+ C
Sample 7				C		C			C								A	T			G		A																													
Sample 8																					G		C																								G	G					+ C
Sample 9													G	T														A	T	A																G	G					
Sample 10							C	G		G	A	C	G		C		A		C						C																					G	G				T		+ C

Example of same multiple sequences from HV1-HV2 Regions that are aligned and presented together.
The Anderson sequence is show on the first box. All deviation from the standard are show as the corresponding letter of the nucleotide which differs from the Anderson.

Figure 2