East Asian mtDNA haplogroup determination in Koreans:

Haplogroup-level coding region SNP analysis and subhaplogroup-level control region sequence analysis

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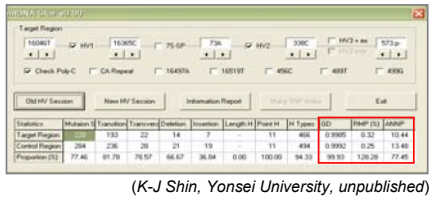
Korean mtDNA database establishment and haplogroup assignment

- A high quality mtDNA control region sequence database was established in 593 Koreans (*http://forensic.yonsei.ac.kr/*)
- Based on shared haplogroup-specific polymorphisms in control region sequence, 592 mtDNAs (99.8%) were classified into various East Asian haplogroups or subhaplogroups
- Statistical parameters were calculated using "mtDNA Star"



(*K-J Shin, Yonsei University, unpublished*)

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mtDNA haplogroup determination has practical value in forensic field

❖ Sequencing and documenting processes are prone to copying errors (e.g. base shift, reference bias, phantom mutations, base misscoring, artifactual recombination)

❖ As mtDNA evolves along a tree, assigning new mtDNA types to a spot in the global mtDNA tree can prevent potential errors in mtDNA database

❖ Phylogenetic analysis is the key tool in understanding the structure of the mtDNA data

The ideal approach is confirmation of diagnostic coding region SNP

❖ Previously identified control region mutation motifs cannot exactly define major haplogroups and their subhaplogroups without complementation of coding region information

❖ As an example, D4, G and M9 mtDNA are not distinguishable only with control region sequence polymorphisms

Sample	Haplogroup	Control region sequence
BF4229	D4	**16223 16362 16519** 73 263 309.1C 315.1C **489** 523d 524d
385	G	**16223 16362 16519** 73 263 309.1C 315.1C **489**
476	G	16078T 16179 **16223 16234 16362** 16519 73 152 263 309.1C 309.2C 315.1C **489**
BF4102	M9	**16223 16234** 16274 **16362** 73 153 263 315.1C **489**
409	G	**16189 16223** 16269 **16278 16362** 73 260 263 284 309.1C 309.2C 315.1C **489**
BF4271	D4	16172Y **16189 16223 16278 16362** 73 263 309.1C 315.1C **489** 573.1C 573.2C 573.3C 573.pC

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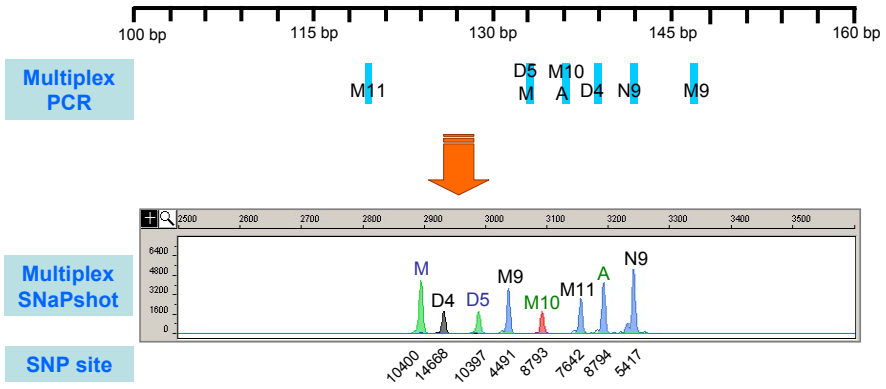
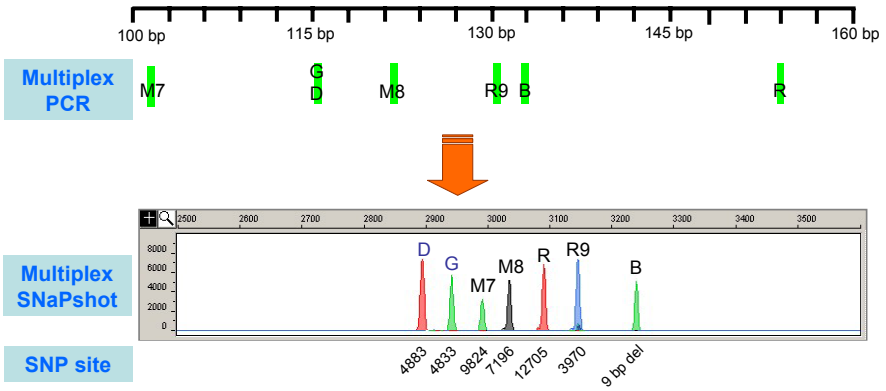
Design of three multiplex systems for coding region SNP scoring

- Multiplex I



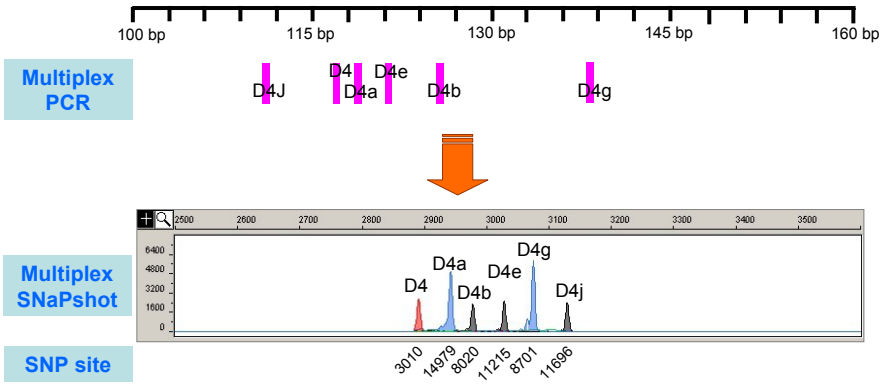
Design of three multiplex systems for coding region SNP scoring

- Multiplex II



Design of three multiplex systems for coding region SNP scoring

- Multiplex III



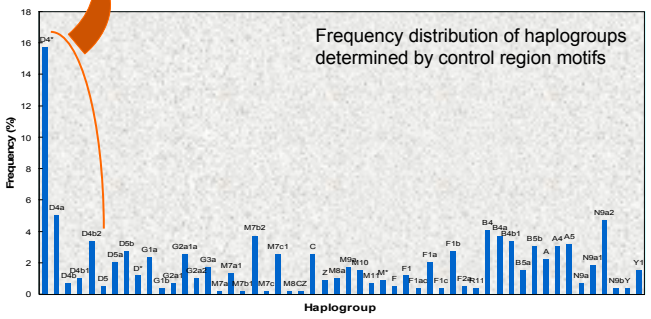
Control region motifs for East Asian haplogroups were identified

Haplogroup	HV1	HV2	HV3 etc
B4	16183C-16189-16217		
B4a	16182C-16183C-16189-16217-16261		(16519), 523d-524d
B4b1	16136-16183C-16189-16217		16519, 499
B4d	16183C-16185-16189d-16217-16234		546
B4c1a	16183C-16189-16217-16311		16519
B4c1b	16140-16183C-16189-16217-16274-16335	150	
B4c1c	16183C-16189-16217-16311	150-195-214	
B4f	16168-16172-16183C-16189-16217-16249-16325	200	16390
B5a1	16129-16140-16187-16189-16266R	93-210	16519, 523d-524d
B5b	16140-16183C-16189-16243		16519, 523d-524d, (or 513d-514d)
A	16223-16290-16319	235	
A4	16223-16290-16319-16362	235	523d-524d
A5	16187-16223-16290-16319	235	523d-524d
N9a	16223-16257A-16261	150	
N9a1	16129-16223-16257A-16261	150	
N9a2	16172-16223-16257A-(16261)	150	
N9a2a	16172-16223-16257A-16261	150	16497
N9b	16183C-16189-16223		16519
Y1b	16126-16231-16266	146	16519
Y2	16126-16231-16311		482

Coding region SNP scoring is useful for molecular dissection of D4 haplogroup

Haplogroup	Freq.(%)
D4*	**8.26**
D4a	5.06
D4b	0.84
D4b1	1.69
D4b2	3.71
D4e	**2.52**
D4g	**1.00**
D4j	**2.35**

Coding region SNP scoring using Multiplex III



Control region motifs for D4 subhaplogroups are identified

Haplogroup	HV1	HV2	HV3 etc
D4	16223-16362		489
D4a	**16129**-16223-16362	**152**	(16519)-489
D4b1	16223-**16319**-16362		489-523d-524d
D4b2*	16223-16362		489-523d-524d
D4b2b	(16223)-16362	**194**	16519-489-523d-524d
D4d	**16245**-16362		489
D4e*	16223-16362		489
D4e1	16223-16362	**94**	489
D4g1	16223-**16278**-16362		489-573.pC
D4h*	16223-16362		489
D4h1	**16174**-16223-16362	**146-183**	489
D4h2	**16174**-16223-**16311**-16362	**152**	489
D4i	16223-**16294**-16362		489
D4j*	16223-16362		489
D4j1	**16184**-16223-**16311**-16362		489
D4k1	**16192**-16223	**195**	489
D4k2	16223-**16274**-**16290**-**16319**-16362	**195**	489
D4m	**16244**-16362		489
D4n	16223-**16355A**-16362		489

Coding region SNP scoring is indispensable in some haplogroups

❖ One of G2a1 haplotype according to control region sequence was found to be D4g haplogroup, and 8 and 1 of D4 haplotypes turned out to be G and M9 haplogroups, respectively

❖ D4 paragroups, e.g., D4*, D4b2*, D4e* and D4j*, which have a mutation motif 16223-16362-489, need coding region SNP scoring for exact haplogroup determination

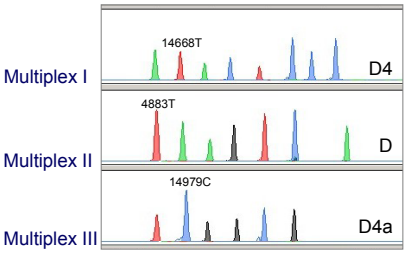
❖ Complementation of coding region SNP information to control region polymorphisms will lead to mtDNA data quality control and molecular dissection of haplogroups

Multiplex systems are proved to be efficient in skeletal remain analysis

❖ Efficiency test was performed in 101 skeletal remains from Korean War (1950~1953) victims

❖ Small amplicon sizes enabled SNP score in old skeletal remains to be successfully analyzed without artifact

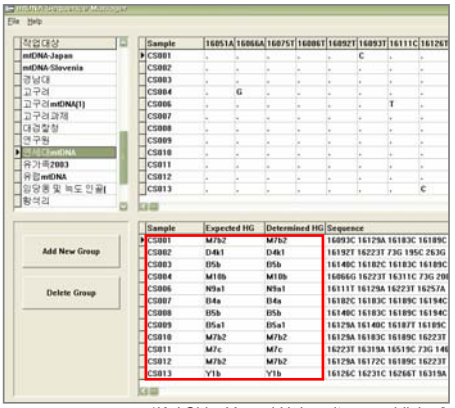
HV1-HV2-HV3 region sequence
16093-**16129**-**16223**-**16362**
73-**152**-263-309.1C-315.1C
489



East Asian HG can be determined using "mtDNA Sequence Manager"

❖ We have developed the haplogroup determining program, "mtDNA Sequence Manager" based on the collated control region mutation motifs for East Asian haplogroups or subhaplogroups

❖ By using this program, 593 Korean mtDNAs and 101 Korean War victim mtDNAs can be classified into various East Asian haplogroups or subhaplogroups



(*K-J Shin, Yonsei University, unpublished*)

Concluding remarks

❖ East Asian haplogroup determination is efficiently carried out using haplogroup-level coding region SNP analysis and subhaplogroup-level control region sequence analysis

❖ Identification of control region mutation motif and molecular dissection of haplogroups can be achieved by coding region SNP analysis

❖ The 3 multiplex systems work well even in degraded samples and it will present a promising means for forensic and human genetics involving East Asian mtDNA haplogroups

Acknowledgement

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