Determination of East Asian Y chromosomal haplogroups using multiplex single base extension reactions

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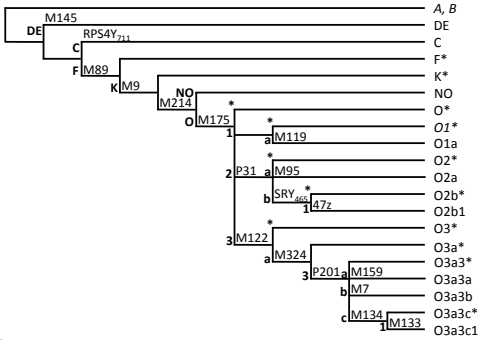
Introduction

Y-chromosomal haplogroup which is defined by the combination of allelic states at hierarchically arranged Y-SNPs and small indels has been studied to infer the origin and the history of evolution and migration of modern human populations. In forensics, Y-SNPs, in spit of its low discrimination power, are used as a marker for the identification of missing persons or mass disaster victims due to its availability for producing small size amplicon which is very useful for the analysis of degraded DNA. Recently, newly revised Y-haplogroup tree containing 311 distinct haplogroups was published and its resolution was increased. Especially haplogroup O, a major haplogroup in East Asia was considerably rearranged. Therefore, more efficient Y-SNP genotyping methods for convenient determination of East Asian Y-haplogroups need to be developed according to the revised Y-haplogroup tree. Here we described three multiplex single base extension reactions for Y-SNP selected hierarchically along the revised Y-haplogroup tree and producing small amplicons. In order to assess their suitability to forensic samples, the multiplexes were validated with sensitivity test on serially diluted DNA and efficiency test on artificially degraded DNA and 10 DNAs extracted from 60-year-old skeletal remains. Finally, a Korean population was analyzed by the developed multiplex systems and the frequencies of Y haplogroups were displayed.

Materials and Methods

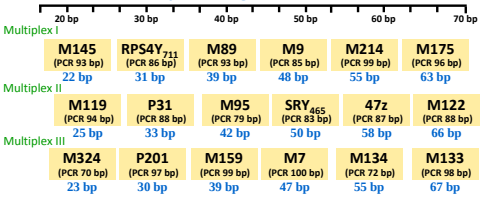
Selection of Y-SNPs from phylogenetic tree



DNA Samples

Three hundred DNAs of unrelated Korean males were obtained from the National Biobank of Korea. Serially diluted DNA samples (1 ng, 500 pg, 250 pg, 125 pg, 62 pg and 31 pg) of 9948 standard DNA (Promega, Madison, MA, USA) were used to detect sensitivity of the multiplexes. Artificially degraded DNA prepared by digesting 1.2 μg of human genomic DNA with 0.02 U of DNase I (NEB, Ipswich, MA, USA) for 40 min and 10 DNA samples extracted from 60-year-old skeletal remains were analyzed to evaluate efficiency of the multiplexes.

Schematic of multiplex single base extension



Multiplex PCR amplification

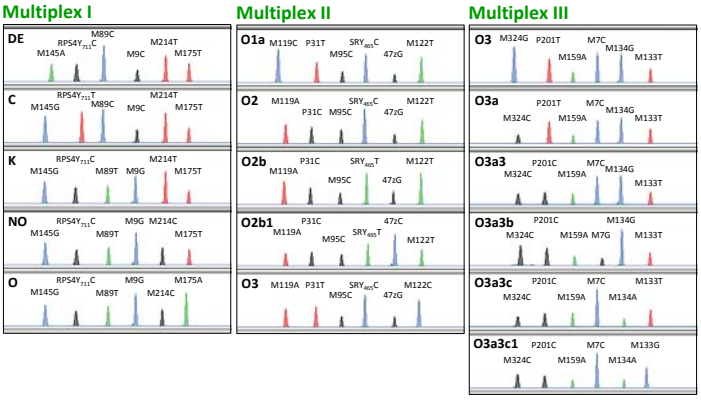
PCR reaction was carried out in a final volume of 25 μl containing 1 ng of template DNA, 2.5 μl of Gold ST*R 10X buffer (Promega) 2.0 U of AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA, USA) and each appropriate concentration of primers.
Cycling condition: 95˚C for 11 min; 33~37 cycles of 94˚C for 20 sec, 60˚C for 1 min, and 72˚C for 30 sec; and a final extension of 72˚C for 7 min
Purification of PCR products: 5 μl of PCR product was purified with 1 U of ExoSAP-IT (USB, Cleveland, OH, USA).

Multiplex single base reaction

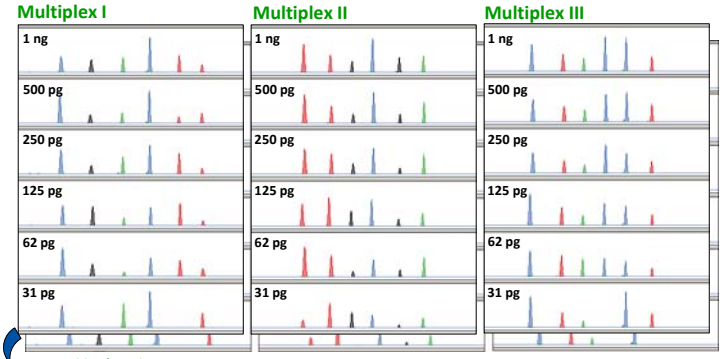
SBE reaction was performed using a SNaPshot™ Multiplex Kit (Applied Biosystems), the purified PCR product and SBE primer mix according to the manufacturer's instructions.
Cycling condition: 25 cycles of 96˚C for 10 sec, 50˚C for 5 sec, and 60˚C for 30 sec
Preparation of SBE products: 1 U of SAP (USB) was added to the extension product to remove unincorporated ddNTP.
Detection system: ABI prism 310 Genetic Analyzer, GeneScan software 3.1 (Applied Biosystems)

Results

Representative electropherograms of each multiplex



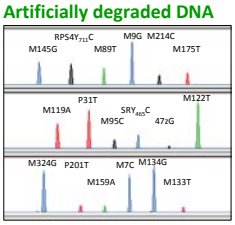
Sensitivity test



All Y-SNPs were successfully called at as low concentration as 62 pg of DNA

Efficiency test

Artificially degraded DNA



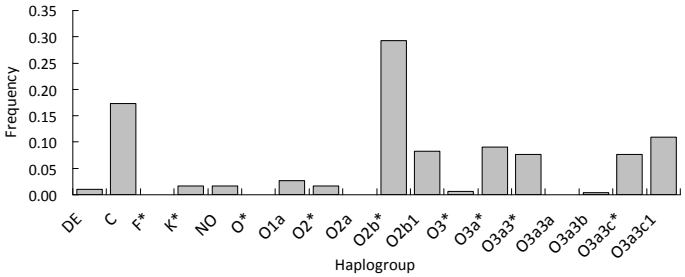
The fully correct Y-SNP profiles were obtained using artificially degraded DNA with the fragment size of around 100 bp.

DNA from old skeletal remain

No.	Concentration (pg/μl)	Success rate (%)		Y haplogroup
		STR	Y-SNP	
1	114.8 ± 10.20	93.3	100.0	NO
2	205.7 ± 2.75	100.0	100.0	O2
3	55.9 ± 5.94	66.7	100.0	NO
4	106.5 ± 3.56	100.0	100.0	O2b
5	766.1 ± 39.03	100.0	100.0	O3a3
6	149.8 ± 11.30	80.0	100.0	O3a3c
7	27.8 ± 0.13	33.3	100.0	O1a
8	169.9 ± 10.96	100.0	100.0	O2b
9	84.9 ± 14.71	100.0	100.0	O3a3
10	275.2 ± 57.04	100.0	100.0	O2b

Some skeletal remain samples had been typed only at some STR loci, Y-haplogroup could be successfully determined based on the amplified Y-SNP scoring results.

Y-haplogroup distribution among 300 Korean males



Conclusion

- **Three multiplex single base extension (SBE) reactions were developed for the identification of Y-haplogroups frequent in East Asians.**
- **The sizes of amplicon were designed to be ranged from 70 to 100 bp to facilitate successful amplification of the degraded DNA.**
- **Validation experiments showed that the amount as small as 62pg of DNA and highly degraded DNA from old skeletal remains can be reliably typed.**
- **Three hundred Korean males were successfully designated to 14 different Y-haplogroups using the multiplex systems**
- **The most common Y-haplogroups observed in Korean population were haplogroup C (17.3%), O2b* (29.3%) and O3a3c1 (11.0%)**
- **These multiplexes will be a useful tool for forensic and evolutionary studies of East Asians**

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