

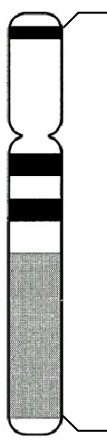
Development of multiplex single base extension reaction and multiplex allele-specific PCR assay to determine East Asian Y chromosomal haplogroups

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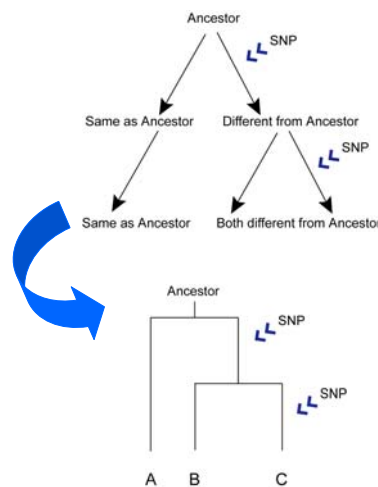
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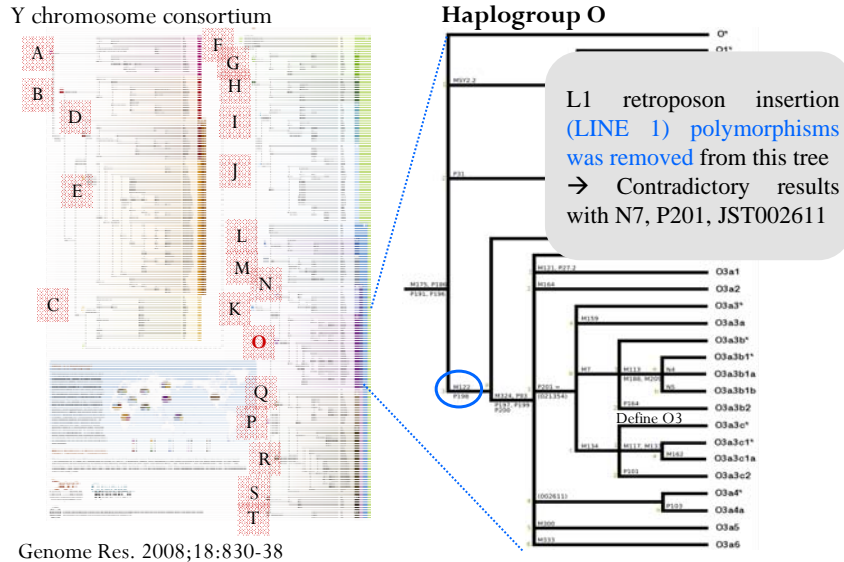
Y chromosomal SNP



Position	Y-SNP	Mutation
2715180	SRY ₄₆₅	C/T
2794854	RPS4Y ₇₁₁	C/T
2881786	M324	G/C
2888196	P201	T/C
3496442	47z	G/C
4138217	M7	C/G
13005251	P31	T/C
13981319	M214	T/C
14018100	M175	-5bp
20175606	M134	-1bp
20175715	M133	-1bp
⋮		
> 600 Y-SNP		



Y chromosome phylogenetic tree



Y haplogroup typing in forensics

Forensic utility of Y-SNPs

- Human identification purposes (criminal, paternity, evolutionary, population studies)
 - Haplogroup typing potential
 - The shared haplogroups with distinctive frequencies in East Asians
- Requiring a sensitive and efficient method for the Y chromosomal haplogroup determination process

Increasing of need for ethnicity prediction

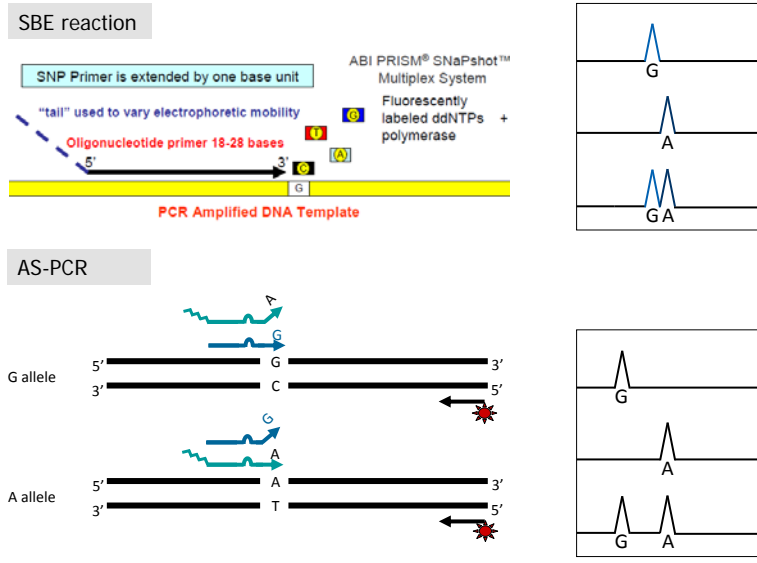
- Globalization of crime suspects and victims
- Increase of movement from East-southern population
- Increase of excavation of Korean War victims and ancient remains.
- Individual identification in mass disaster such as airplane crashes, tsunamis or terrorist attacks where people from various geographical areas are involved

Objects

- Development of multiplex systems for determination of East Asian Y haplogroups
 - **Single base extension (SBE)** method with SNaPshot™ Multiplex kit
 - Amplicon size < 100 bp to be suitable for the analysis of **highly degraded forensic samples**
 - **Allele specific PCR (AS-PCR)** assay with fluorescent dyes
 - Convenient method similar to STR typing for **reference samples**
- Validation of the multiplex systems
 - Sensitivity test and efficiency test
 - Concordance test between multiplex SBE reaction and multiplex AS-PCR assay



Strategy for SNP typing



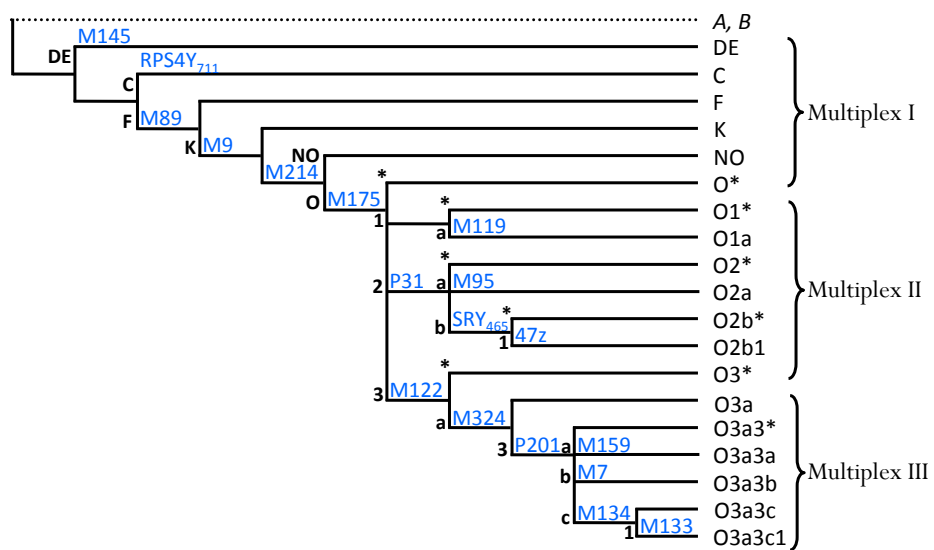
Materials and Methods

□ Detection Systems

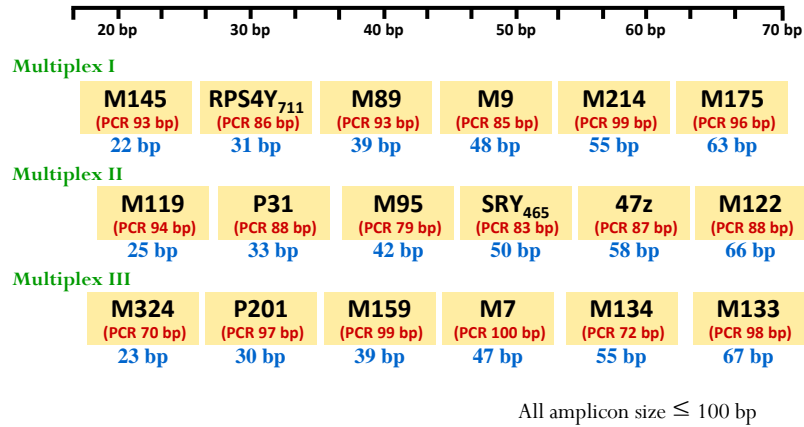
- ABI prism 310 genetic analyzer, GeneScan software 3.7, and Genemapper 3.2 software (Applied Biosystems)



Selection of Y-SNP for Multiplex SBE reactions

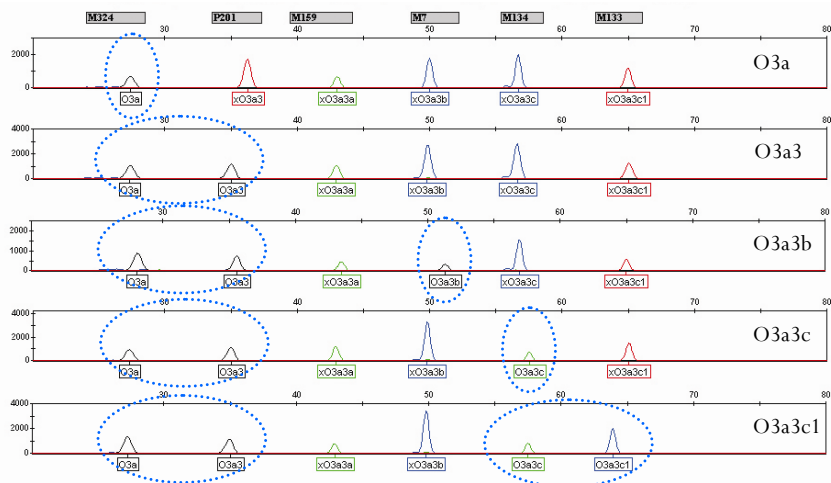


Schematic of multiplex SBE reactions

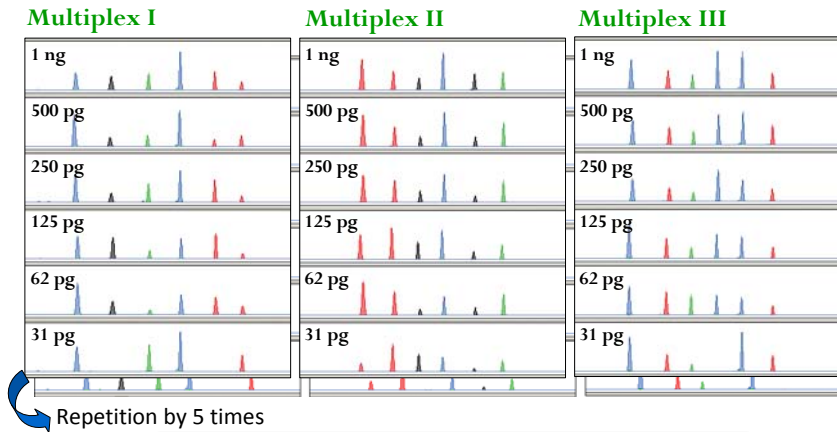


Y haplogroup determinations using multiplex SBE reactions

Multiplex III



Sensitivity test



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

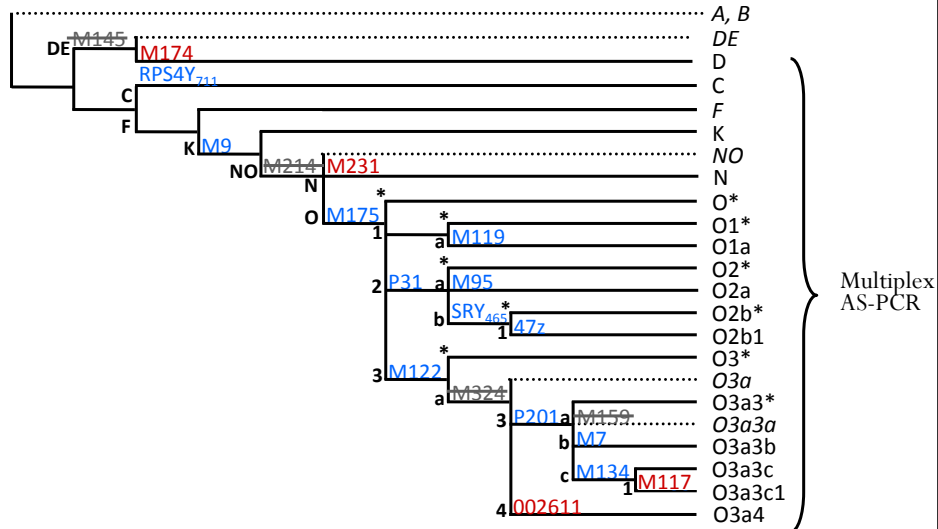
Efficiency test

DNA from old skeletal remains

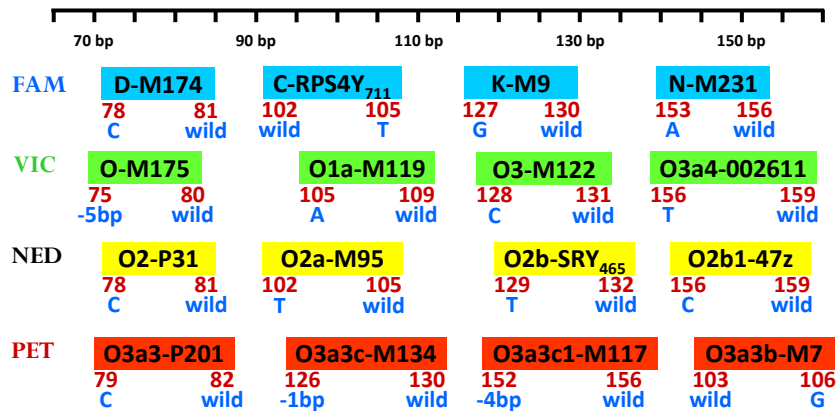
No.	Concentration (pg/ μ l)	Success rate (%)		Y haplogroup
		STR	Y-SNP	
1	114.8 \pm 10.20	93.3	100.0	NO
2	205.7 \pm 2.75	100.0	100.0	O2
3	55.9 \pm 5.94	66.7	100.0	O2b
4	106.5 \pm 3.56	100.0	100.0	O2b
5	766.1 \pm 39.03	100.0	100.0	O3a3
6	149.8 \pm 11.30	80.0	100.0	O3a3c
7	27.8 \pm 0.13	33.3	100.0	O1a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

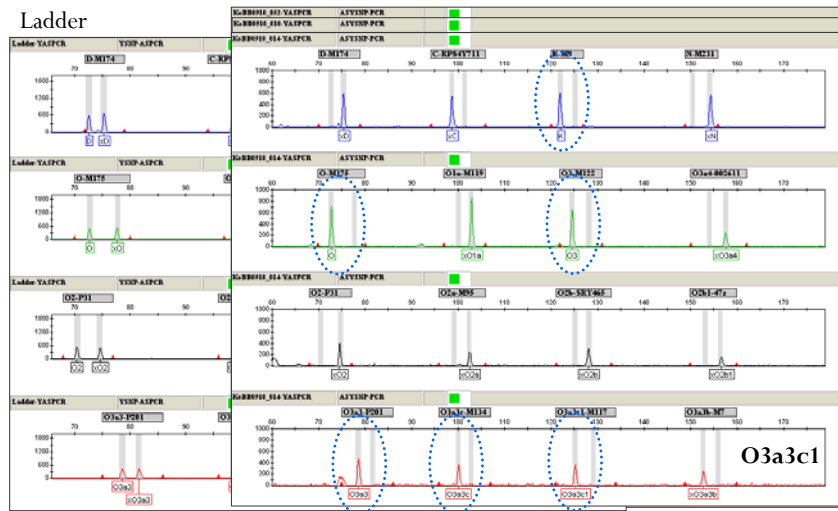
Selection of Y-SNP for AS-PCR assay



Schematic of multiplex AS-PCR



Haplotyping using multiplex AS-PCR assay



Future study for multiplex AS-PCR assay

□ Concordant test

- A total of 300 Korean males will be tested by this assay and the results will be compared with those from the multiplex SBE reactions

□ Validation test

- Sensitivity of the multiplex AS-PCR assay
- Efficiency of the multiplex AS-PCR assay

Conclusion

- ❑ Two different multiplex PCR sets, [three multiplex SBE reactions](#) and a [multiplex AS-PCR assay](#), were developed for the identification of Y-haplogroups frequent in East Asians for analyzing [forensic samples](#) and [reference samples](#), respectively.
- ❑ Using the multiplex SBE reactions, reliable genotypes were obtained from the amount as 62 pg of DNA and highly degraded DNA from old skeletal remains.
- ❑ The multiplex SBE reactions are very sensitive and optimized for analyzing old degraded forensic casework samples.
- ❑ The multiplex allele-specific PCR assay was developed for simple, rapid and reliable scoring of alleles in large number of samples and will be tested concordance with the results of the multiplex SBE reactions.
- ❑ This study would suggest that selective use of these multiplex sets for specific purpose is useful to obtain genotypes rapidly and effectively in forensic casework and reference samples.



Thank you for your attention



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