



# Search for Missing Casualties of the Korean War: Systematic Approaches to DNA Typing of Old Skeletal Remains

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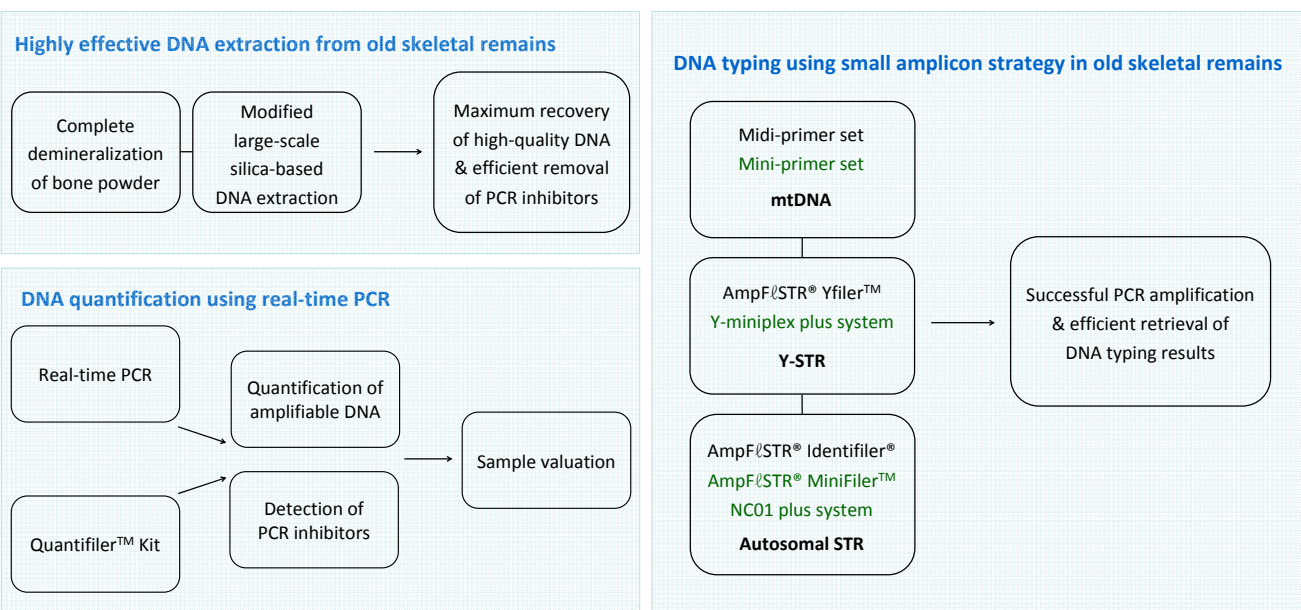
## Introduction

Since the year 2000, the identification of missing casualties of the Korean War (1950-1953) have been performed using mitochondrial DNA (mtDNA) profiles. However, recent advances in DNA extraction techniques and small amplicon approaches have significantly increased the possibility of obtaining DNA profiles from highly degraded old skeletal remains. Therefore, 21 skeletal remains of Korean War victims and 24 samples from biological relatives of the supposed victims were selected based on circumstantial evidence and/or mtDNA matching results, and analyzed by employing a recently developed method for efficient DNA extraction and generating autosomal short tandem repeat (STR) and Y-STR results using three commercial STR systems (AmpF $\ell$ STR<sup>®</sup> Identifier<sup>®</sup>, AmpF $\ell$ STR<sup>®</sup> MiniFiler<sup>™</sup> and AmpF $\ell$ STR<sup>®</sup> Yfiler<sup>™</sup>) and two in-house mini-STR systems (the minplex NCO1 plus and Y-miniplex plus systems) to confirm alleged relationships.

## Typical Workflow of DNA Typing of Old Skeletal Remains



## Recent Systematic Approaches to DNA Typing of Old Skeletal Remains



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**Table 1** Twenty one skeletal remains of Korean War victims and 24 samples from biological relatives of the supposed victims were selected based on circumstantial evidence and/or mtDNA matching results, and cumulative log likelihood ratios were calculated from serially added autosomal STR, Y-STR and mtDNA analysis results

No.	Skeletal sample	Family sample	Alleged family relationship	Log Likelihood Ratio						
				AmpF $\ell$ STR <sup>®</sup> Identifier <sup>®</sup>	AmpF $\ell$ STR <sup>®</sup> MiniFiler <sup>™</sup>	NCO1 plus	AmpF $\ell$ STR <sup>®</sup> Yfiler <sup>™</sup>	Y-miniplex plus	mtDNA	
1	SR0001	F50011	brother	-1.6	1.5	0.4	1.8	2.8	5.1	
2	SR0002	F50001	brother	3.0	3.2	2.4	4.9	4.9	6.9	
		F50001-1	sister	6.0	6.9	6.5	—	—	8.4	
3	SR0004	F50016	sister	1.3	4.0	5.7	—	—	7.7	
4	SR0008	F50009	brother	7.7	7.7	7.0	5.8	5.8	8.1	
5	SR0011	F50016	sister	2.6	4.4	5.6	—	—	7.6	
6	SR0012	F50006	brother	1.4	1.2	2.7	4.7	5.0	7.3	
7	SR0014	F50015	brother	-2.4	-2.3	-3.6	-4.5	-4.3	-2.0	
8	SR0015	F50012	brother	2.5	5.5	6.4	8.1	8.2	10.5	
9	SR0016	F50007	brother	0.4	4.3	6.1	8.5	8.5	10.8	
10	SR0017	F50003	brother	2.0	3.9	4.5	6.9	6.9	9.1	
11	SR0018	F50013	sister	3.6	3.9	4.4	—	—	6.7	
12	SR0078	F57221	brother	1.8	3.5	4.9	6.8	7.0	9.0	
13	SR0079	F57216	sister	3.3	5.0	6.5	—	—	8.7	
		F57217	nephew	2.7	3.0	2.8	5.4	5.4	—	8.7
14	SR0080	F57222	brother	0.6	5.9	6.4	9.0	9.0	11.4	
15	SR0081	F57218	brother	1.9	4.6	5.0	7.6	7.6	10.1	
16	SR0083	F57219	son	2.7	3.1	3.7	5.6	6.2	—	
		F57220	daughter	2.4	4.0	5.0	—	—	—	
17	SR0135	F57205	brother	1.0	4.4	4.4	7.0	7.0	9.1	
		F57206	sister	1.2	3.1	3.5	—	—	5.7	
18	SR0221	F57227	brother	3.5	5.6	6.0	8.2	8.2	10.5	
19	SR0315	F50019	sister	1.3	1.3	3.1	—	—	5.4	
20	SR4003	F50020-1	son	3.3	3.3	3.8	6.3	6.3	—	
21	SR7001	F57001	sister	0.6	0.6	0.2	—	—	2.6	

## Conclusion

- Optimization of DNA extraction step by employing large scale silica-based extraction method combined with complete demineralization could considerably enhance the genotyping success rate in old skeletal remains.
- By using reduced-size amplicons of the AmpF $\ell$ STR<sup>®</sup> MiniFiler<sup>™</sup> Kit and in-house miniplex NCO1 plus as a complement to the AmpF $\ell$ STR<sup>®</sup> Identifier<sup>®</sup> kit, all 21 skeletal remain samples produced successful STR typing results with the mean success rate of 95.8% (17.2 from 18 loci). Based on autosomal STR genotyping results, likelihood ratios (LR) for the alleged relationship were over 100 in almost all cases except for 3.
- By using reduced-size amplicons of in-house Y-miniplex plus as a complement to the AmpF $\ell$ STR<sup>®</sup> Yfiler<sup>™</sup> Kit, Y-STR genotyping efficiency was significantly increased displaying the mean success rate of 84.1% (14.3 from 17 loci). In case of having paternal male relatives, Y-STR typing confirmed the alleged relationship by increasing the LRs.
- Cumulative LR obtained from autosomal STR, Y-STR, and mtDNA results confirmed the alleged relationship between missing persons and their relatives with great probabilities.
- Our analyses emphasize the value of mini-STR and Y-STR systems as well as efficient DNA extraction method in the success and efficiency of forensic DNA testing for the identification of old skeletal remains.