Improved Y-STR analysis of degraded DNA using reduced size STR amplicons

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Introduction

Y-STR markers have become a valuable tool for forensic DNA typing especially in male sibship analysis and sexual crime investigation. However, in many forensic cases, DNA samples are highly degraded and environmental contaminants are commingled with specimen. Even with commercially available Y-STR kits, the poor amplification of the larger sized loci is generally observed in such cases. Meanwhile, it has been reported that the use of SNPs or miniSTR markers can increase the probability of a degraded sample to be typed. Therefore, we developed the two new multiplex PCR sets for 17 Y-STR loci (DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, GATA C4 and GATA H4) by reducing the sizes of some amplicons in a commercial Y-STR kit, AmpF*l*STR® Yfiler™ (Applied Biosystems), tested their efficiency and compared these systems with AmpF*l*STR® Yfiler™ using enzymatically degraded DNA and DNAs obtained from 50-year old skeletal remains.

Materials and Methods

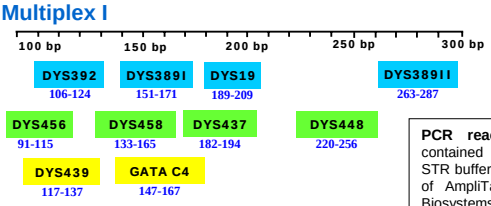
DNA samples

Serially diluted DNA samples (1 ng, 500 pg, 250 pg, 125 pg, 62 pg, 31 pg and 15 pg) of the control 9948 male DNA (Promega, Madison, MA) were used to detect sensitivity of Y-STR systems. Degraded DNA was prepared by enzymatically digesting 3.0 μg of the extracted blood DNA with 0.01 U DNase I (NEB, Beverly, MA) for time periods of 0, 2, 5, 10, 15, 20 and 30 min. Also, 30 DNA samples (>0.01 ng/μl) were extracted from 50-year old skeletal remains from Korean War victims by modification of the QIAamp protocol (Qiagen, Hilden, Germany) by Yang *et al.*

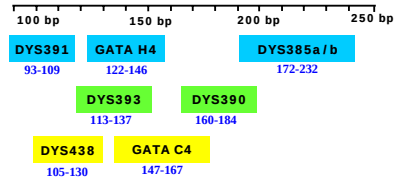
Primer design

PCR primers were designed against each reference sequence using web-based Primer3 (www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The size of each amplicon was made as small as possible around target region that consisted of the Y-STR repeat. The independently selected candidate primer pairs were screened for potential secondary structure using AutoDimer software (www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage/AutoDimerProgramHomepage.htm). And then, among those set of primers, the primer pairs which showed high PCR efficiency and no interference with the other primers were finally selected.

Multiplex I



Multiplex II



PCR reaction: Total 10 μl reaction contained 1~2 μl of DNA, 1.6 μl of Gold STR buffer (Promega, Madison, WI), 2.5 U of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA), and each appropriate concentration of primers

Cycling condition: 95°C 11 min; 96°C 60 sec; 94°C 30 sec, 60°C 30 sec, 70°C 45 sec x 10; 90°C 30 sec, 60°C 30 sec, 70°C 60 sec x 20~25; 60°C 45 min using the GeneAmp 9600

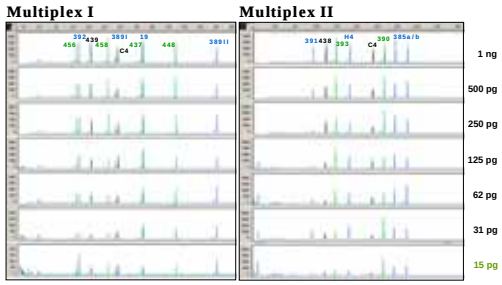
Detection system: ABI prism 310 Genetic Analyzer, GeneScan software 3.1 and Genotyper 2.5 software (Applied Biosystems, Foster City, CA)

Information of 17 Y-STRs examined in this study

Y-STR Locus	GenBank Accession	9948 DNA Allele	Allele Range	Product Size AmpF*l*STR®Yfiler™	Product Size Mini-Y Multiplex	Size Reduction
Multiplex I						
DYS392	AC011745	13	10-16	303-321 bp	106-124 bp	197 bp
DYS389I	AF140635	13	11-16	150-170 bp	151-171 bp	-
DYS19	AC017019	14	13-18	187-207 bp	188-208 bp	-
DYS389II	AF140635	31	26-32	262-286bp	263-287 bp	-
DYS456	AC010106	17	12-18	99-123 bp	91-115 bp	-
DYS458	AC010902	18	13-21	133-165 bp	133-165 bp	-
DYS437	AC002992	15	13-16	182-194 bp	182-194 bp	-
DYS448	AC025227	19	17-23	282-318 bp	220-256 bp	62 bp
DYS439	AC002992	12	9-14	204-224 bp	117-137 bp	87 bp
GATAC4	G42673	23	19-24	242-262bp	147-167 bp	95 bp
Multiplex II						
DYS391	AC011302	10	7-9	152-168 bp	93-109 bp	59 bp
GATAH4	AC011751	12	8-14	122-146 bp	122-146 bp	-
DYS385a/b	Z93950	11,14	8-23	247-307 bp	172-232 bp	75 bp
DYS393	AC006152	13	10-16	115-139 bp	113-137 bp	-
DYS390	AC011289	24	21-27	209-233 bp	160-184 bp	49 bp
DYS438	AC002531	11	9-14	228-253 bp	105-130 bp	123 bp

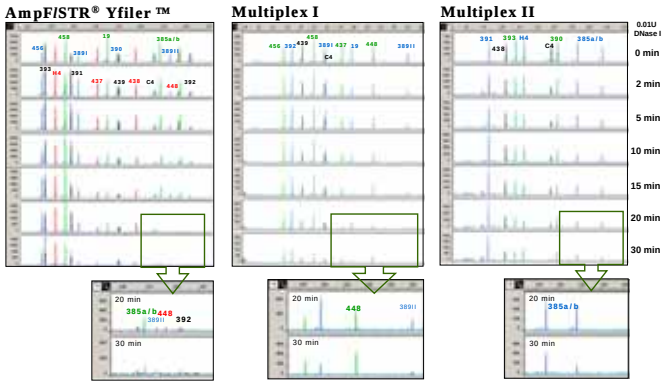
Results

Sensitivity study



Full profiles were obtained with ≥ 31 pg of 9948 male DNA at 33 cycles of thermal amplification. At lower concentration of template DNA, allele drop-out was most commonly observed at DYS19.

Amplification of enzymatically degraded DNA



As the extent of DNA degradation increased with enzymatic digestion, PCR yield for the larger sized loci began to decrease. However, the mini-YSTR multiplex sets were capable of producing more complete profiles than the AmpF*l*STR® Yfiler™ kit.

Efficiency test in 30 DNA samples from Korean War victims

Amplification rates in reduced size Y-STRs

	AmpF*l*STR® Yfiler™	Mini-YSTR Multiplex
DYS392	9 (30.0%)	24 (80.0%)
DYS438	11 (36.7%)	20 (66.7%)
GATA C4	14 (47.0%)	28 (93.0%)
DYS439	12 (40.0%)	21 (70.0%)
DYS385a/b	18 (60.0%)	23 (76.7%)
DYS448	12 (40.0%)	20 (67.0%)
DYS391	18 (60.0%)	27 (90.0%)
DYS390	14 (46.7%)	18 (60.0%)

Amplification rates in unreduced size Y-STRs

	AmpF*l*STR® Yfiler™	Mini-YSTR Multiplex
DYS456	24 (80.0%)	30 (100%)
DYS393	21 (70.0%)	24 (80.0%)
GATA H4	20 (66.7%)	22 (73.3%)
DYS458	23 (76.7%)	26 (86.7%)
DYS437	19 (63.0%)	20 (67.0%)
DYS389I	23 (77.0%)	21 (70.0%)
DYS19	13 (43.0%)	17 (57.0%)
DYS389II	17 (57.0%)	16 (53.0%)

	AmpF*l*STR® Yfiler™	Mini-Y Multiplex	AmpF*l*STR® Yfiler™ + Mini-Y Multiplex
Amplified Loci	279 (54.7%)	374 (73.3 %)	394 (77.3 %)

Conclusion

- Redesigned Y-STR primer sets, in which amplicon size is kept at minimum, provide an effective tool for degraded forensic samples.
- The combined use of these primer sets with commercial kits will increase the probability that degraded samples can be typed.
- They can be used to check the presence of allele drop-out which occurs by primer binding site mutation.
- Although a few Y-STR loci cannot be made into smaller amplicon, development of additional reduced size Y-STR system is required to increase discrimination power of Y-STRs in degraded forensic samples.

References

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3.Yang D.Y., Eng B., Waye J.S., Dudar J.C., Saunders S.R. Improved DNA extraction from ancient bones using silica-based spin columns, Am J Phys Anthropol, 105; 539-43, 1998.