A Prototype Y-STR Multiplex PCR System to Improve DNA Typing Performance with Forensically Relevant Samples

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Introduction

Y chromosomal short tandem repeats (Y-STRs) are generally analyzed in forensic casework, such as sexual assaults, paternity test and male lineage investigation. However, DNA samples from forensic case are often degraded and/or tainted under the harsh condition. When such low quality DNA is used for STR typing, the poor genotyping results are observed with larger-sized amplicons even with a well-made commercial kit, for example, AmpFlSTR® Yfiler™PCR Amplification kit (Applied Biosystems, Foster City, CA, USA). To encourage better Y-STR analysis, the complementary multiplex PCR system with reduced size amplicons is necessary. In this regard, we present a prototype Y-STR multiplex PCR system, Kplex Y17 system, which was designed to improve DNA typing performance on various forensically relevant samples.

Materials and Methods

DNA samples

1 ng of standard DNA 2800M (Promega, Madison, WI, USA) was used to optimize a condition of multiplex PCR, and serially diluted DNA samples (500 pg, 250 pg, 125 pg, 62.5 pg and 31.2 pg) of standard DNA 2800M were used to detect sensitivity of the multiplex PCR system. Mixed DNA templates, which were comprised of 100 pg of male control DNA 007 mixed with female DNA in the ratio of 1:0, 1:1, 1:10, 1:100 and 1:1000, were used for specificity test.

Primer design for PCR amplification

We collected the information of 17 Yfiler STRs from STRBase (http://www.cstl.nist.gov/biotech/strbase) and GenBank (http://www.ncbi.nlm.nih.gov/genbank), and designed proper primers for PCR amplification using Primer3 (http://frodo.wi.mit.edu/primer3/input.htm).

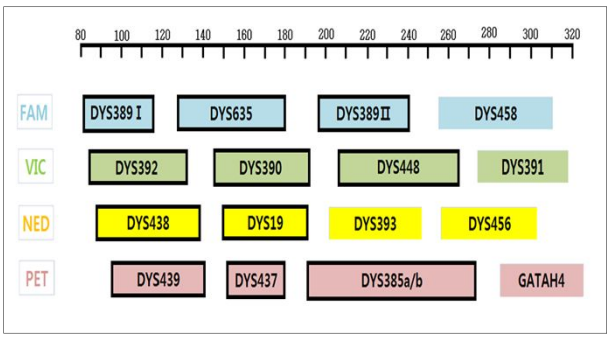
Multiplex PCR and genotyping

Multiplex PCR : The multiplex PCR was performed in a final volume 10 μl that contained 1 ng of template DNA, 1.0 μl of Gold ST*R 10x Buffer (Promega) 3.0 U AmpliTaq Gold® DNA polymerase (Applied Biosystems) and appropriate concentrations of primers. Thermal cycling was performed under the following conditions : 95°C for 11 m; 30 cycles of 94°C for 20 s, 60°C for 90 s, 72°C for 60 s; and a final extension of 60°C for 45 min.

Genotyping : Capillary electrophoresis was performed on automatic DNA sequencers, ABI PRISM 310 and 3130*x*l Genetic Analyzer (Applied Biosystems). Bin set and Panel were constructed for Y-STR genotyping using GeneMapper ID Software versions 3.2 (Applied Biosystems).

Results

Fig. 1. Schematic of multiplex PCR system for analysis of 17 Y-STRs



Amplicon sizes of the markers in black box are smaller than those of the Yfiler kit

Table 1. Kplex Y17 system loci, alleles and amplicons size range

Locus	Allelic range	Amplicon size range (bp)		Size difference*
		Kplex Y17	AmpFlSTR Yfiler™	
DYS389 I	9-17	81-113	139-173	-59
DYS635	15-28	128-180	226-278	-98
DYS389 II	24-35	196-240	253-298	-57
DYS458	10-24	255-311	115-172	+140
DYS392	4-20	84-133	282-333	-199
DYS390	17-29	143-191	188-236	-45
DYS448	14-24	203-264	262-325	-60
DYS391	5-16	273-317	143-188	+130
DYS438	6-16	86-136	214-264	-128
DYS19	9-19	148-188	172-211	-24
DYS393	7-18	200-244	96-140	+104
DYS456	11-23	254-302	96-144	+158
DYS439	6-17	94-138	190-233	-96
DYS437	11-18	149-177	175-202	-26
DYS385a/b	7-28	189-274	243-330	-55
GATAH4	8-18	285-325	122-162	+163

* Amplicon size difference between the Kplex Y17 and the Yfiler kit

Fig. 2. Electropherogram of Kplex Y17 PCR products using 1 ng of standard DNA 2800M

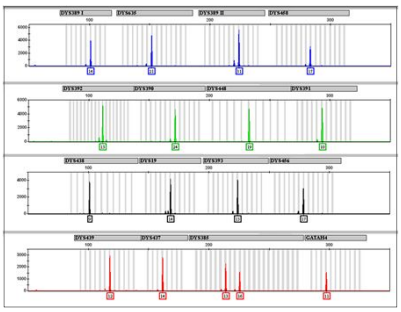


Fig. 3. Electropherogram of sensitivity test using serially diluted DNA

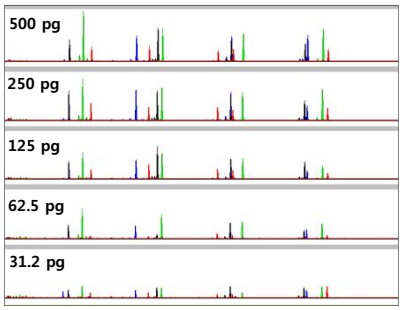
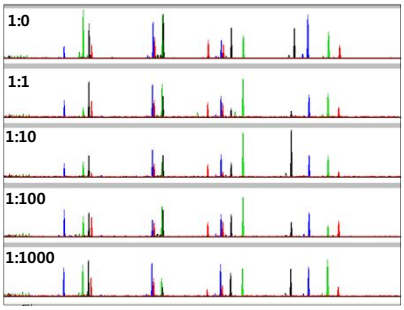


Fig. 4. Electropherogram of specificity test using mixed male/female DNA in various ratios



Conclusions

- The amplicon sizes of large-sized (> 200 bp) Yfiler loci including DYS389I/II and DYS385a/b were reduced to mini- or midi-sizes in the Kplex Y17.
- The Kplex Y17 system showed sensitivity to get full profile from 100 pg male DNA and specificity to analyze male DNA profile from mixed male/female DNA in the ratio of 1:1000 without any interference or reduction of signal.
- Allelic size range was expanded to cover most of the off ladder alleles reported in Koreans and the PowerPlex® Y23 system (Promega), and the interval between loci was adjusted to be at least 10 bp even in case rare allele exists.
- Therefore, the Kplex Y17 system will be a real complement to the Yfiler kit by enabling researchers to obtain DNA full profiles even at large-sized loci of Yfiler with challenged samples.

Acknowledgement

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