Internal Validation of the Short Amplicon Y STR multiplex system for use in forensic casework

검찰 PROSECUTION SERVICE

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Abstract

Y-chromosome short tandem repeat(Y-STR) markers are being used as tools for distinguishing male DNA as is present in many sexual assault samples. DNA samples from forensic case, however, are often degraded and/or tainted by environmental contaminations. To increase the success rate of Y-STR genotyping for degraded forensic sample. We had developed new Y-miniplex system (DYS391, DYS439, DYS385, DYS392, DYS390, DYS438, DYS635) in previous research(M.J. Park, K.J. Shin, 2007). In this study, we have performed internal validation study of new Y-miniplex system to implementing into routine forensic casework analysis.

In a concordance study between the commercial Y-STR kit and the new Y-miniplex system, no genotype differences were revealed in 100 randomly selected male individuals.

A sensitivity test using serially diluted standard 9948 male DNA showed that all the values of loci in the Y-miniplex were reliable at template concentrations as low as 30 pg. In the male-male mixtures, a complete profile from the minor component was detected up to 1:16 ratio. Complete Y-STR profiles were obtained when 30pg male DNA was mixed with female DNA at ratios up to 1:8000. According to results from the test on degraded and tiny amount of Forensic DNA samples(Old bone & Rape case sample), new Y-miniplex system was proved to be a quite effective tools for analyzing forensic DNA samples. We conclude that the new Y-miniplex system appears to be a supplement tool in forensic practices of degraded forensic casework samples with other commercial kits.

Materials and Methods

The PCR amplification was performed in a reaction volume of 10 ㎕ composed of 0.3 ㎕ (1.5 units) Of AmpliTaq Gold® DNA polymerase (*Applied Biosystems*), 1 ㎕ of Gold ST*R 10X Buffer (*Promega*), 1 ㎕ of 10X primer mix and 0.1~0.5ng target DNA. The standard thermal cycling conditions consisted of enzyme activation at 95℃ for 11 minutes, followed by 30 cycles of denaturation at 94℃ for 20 sec, annealing at 59℃ for 2 min, and extension at 72℃ for 1min. A final extension was performed at 60℃ for 45 min . 1.5 ㎕ of PCR product was mixed with 12 ㎕ of Hi-Di™ Formamide and 0.15 ㎕ of GeneScan ™ 500 LIZ® size standard (*Applied Biosystems*) . Separation and detection of PCR products were accomplished with ABI Prism® 3130*xl* Genetic Analyzer 16-capillary array system (*Applied Biosystems*) according to the manufacture ś recommendation. Following data collection, samples were analyzed using Gene Mapper ID v3.2 software (*Applied Biosystems*).

Results

Table 1.2 Information on Y-miniplex system ; Stutter values and Size Reduction.

Y-STR Loci	Allele Range AmpFlSTR Yfiler	Allele Range Y-miniplex	Peak Height (RFU)** AmpFlSTR Yfiler	Peak Height (RFU)** Y-miniplex	Size* Reduction(bp)
DYS391	7 - 13	7 - 13	2978	2557	59
DYS439	8 - 15	8 - 15	2570	5901	89
DYS385 a/b	**7 - 25**	**9 - 22**	2806/2353	4117/3668	75
DYS390	7 - 18	7 - 18	3133	4236	197
DYS392	18 - 27	18 - 27	2406	3975	49
DYS438	8 - 13	8 - 13	2701	4996	119
DYS635	20 - 26	20 - 26	2129	4622	95

* M.J. Park, K.J. Shin, Int. J. Legal Med (2007)

** Average Peak Height (RFU) from 100 male samples(0.1ng/ul) were recorded.

STR Loci	Stutter Range %	Mean Stutter*	SD	Upper Range Stutter %
DYS391	4.7 - 10.8	**6.6**	0.9	9.3
DYS439	3.6 - 11.6	**6.1**	1.6	10.9
DYS385 a/b	3.0 - 13.4	**5.4**	1.9	11.1
DYS390	3.1 - 10.6	**7.4**	1.1	10.7
DYS392	5.8 - 17.0	**9.6**	1.7	14.7
DYS438	1.3 - 4.9	**2.7**	1.2	6.3
DYS635	3.7 - 10.9	**6.5**	1.4	10.7

*The stutter values from 100 male samples(0.1ng/ul) were recorded

Upper Range Stutter % = Mean Stutter + 3 S.D(Standard Deviation.).

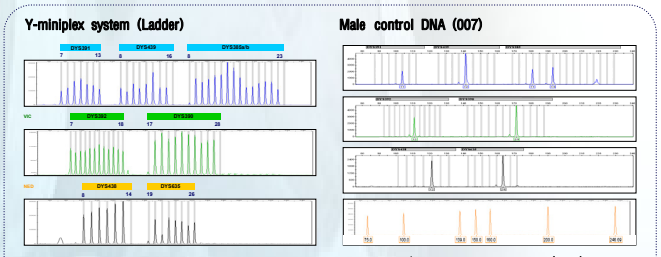

Figure 1. Y-miniplex system ladder and Profile of 0.1 ng/ul Male control DNA (007).

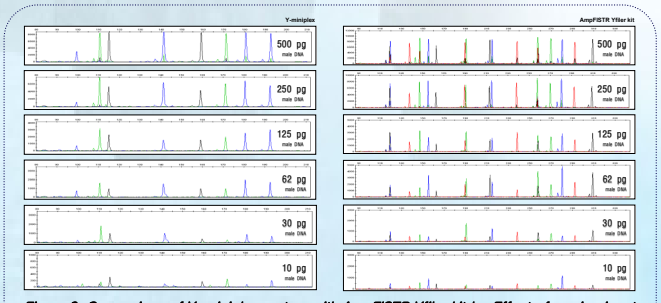

Figure 2. Comparison of Y-miniplex system with AmpFlSTR Yfiler kit I : Effect of varying inputs of template 9948 male DNA (500pg- 10pg)on intracolor peak height.

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Results

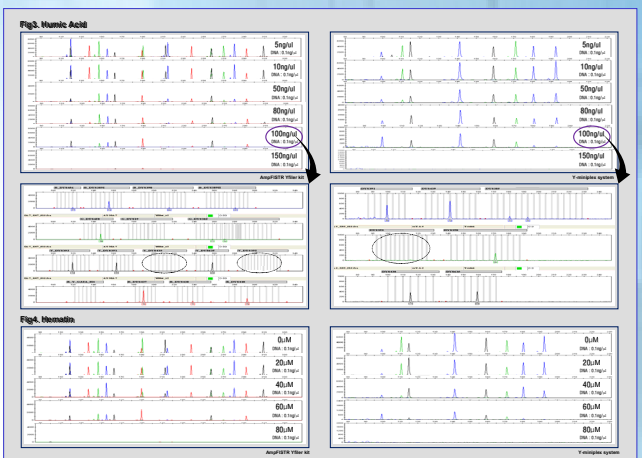


Figure 3. 4. Comparison of Y-miniplex with AmpFlSTR Yfiler kit II.

: DNA amplified with the Y-miniplex in the presence of varying concentrations of PCR Inhibitors (humic acid and hematin).

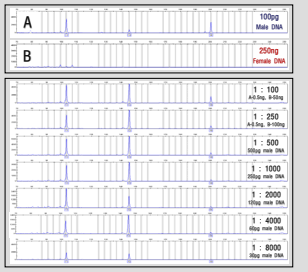


Figure 5. Male-Female Mixture Study in Y-miniplex.

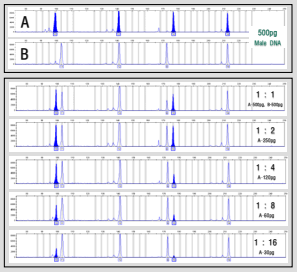


Figure 6. Male-Male Mixture Study in Y-miniplex.

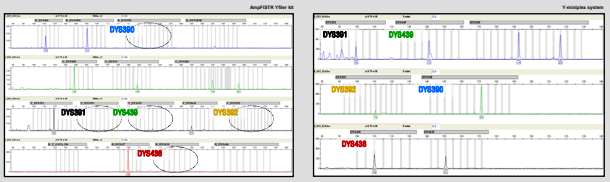
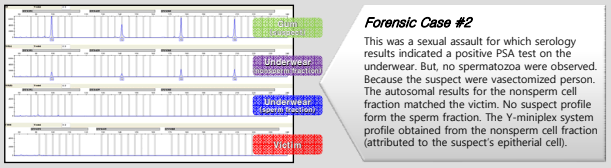


Figure 7. Comparison of Y-miniplex with ABY-filer III : Forensic Case #1.

Complete profiles were get for Old pelvis bone sample(20years) in Y-miniplex.



Forensic Case #2

This was a sexual assault for which serology results indicated a positive PSA test on the underwear. But, no spermatozoa were observed. Because the suspect were vasectomized person. The autosomal results for the nonsperm cell fraction matched the victim. No suspect profile form the sperm fraction. The Y-miniplex system profile obtained from the nonsperm cell fraction (attributed to the suspect's epitherial cell).

Figure 8. Complete profiles were obtained for Vasectomy suspect's semen in Y-miniplex.

Conclusions

- Validation studies were following SWGDAM guidelines.
- Y-miniplex can provide fully concordant results to commercial STR kit in the NIST SRM 2395 and the 100 Korean male samples.
- Y-miniplex proved to be a useful tool that can produce a better signal from degraded DNA than the commercial Y-STR kit. (humic acid test, old bone case)
- Y-miniplex system is useful for typing forensic sample which male to male mixed sample and low levels of male DNA in the presence of high levels of female DNA.
- The new Y-miniplex system appears to be a supplement tool in forensic practices with other commercial kits.

Reference

1. John M Butler(2003) The Development of Reduced Size STR Amplicons as Tools for Analysis of Degraded DNA, J. Forensic Sci. 48, 1054-1064.
2. Julio J. Mulero.(2006) Development and Validation of the AmpFlSTR® Yfiler™ PCR Amplification Kit : A Male Specific, Single Amplification 17 Y-STR Multiplex, J. Forensic Sci. 51, 64-74.
3. Myung Jin Park(2007) Y-STR analysis of degraded DNA using reduced-size amplicons, Int. J. Legal Med., 121, 152-157.
4. Ann Marie Gross.(2008) Internal validation of the AmpFlSTR Yfiler™ Amplification Kit for Use in Forensic Casework., J. Forensic Sci. 53, 125-134.