A self developed multiplex PCR system for analysis of CODIS 13 STR loci used in Korean Criminal Database

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Background

- Launching of Korean Criminal DNA Database in 2010 led us perform analysis of huge samples
- Multiplex PCR depend on commercial kits which were developed in foreign country
- It needs high costs which accounts for nearly all of the analysis costs

➡ **Self developed multiplex PCR system**

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Objects

- Construction of self developed multiplex PCR system for 13 CODIS STRs and Amelogenin
- Smaller amplicon than Identifiler® or PowerPlex®16 for each locus
- Inclusion of rare allele observed in Korean population

Material & Methods

- Sequence information: GenBank
- Primer design (http://frodo.wi.mit.edu/primer3)
- Monoplex PCR → Multiplex PCR
- PCR condition

Reaction mix	Volume (㎕)
dH_2O	5.6
10 x Gold STR buffer	1.0
5 x Primer mix	2.0
Gold Taq Polymerase (5U/㎕)	0.4
Template DNA (1ng/㎕)	1.0
Total	10.0

Thermal cycle (= Identifiler®)

95°C	11 min	
94°C	1 min	
59°C	1 min	x 28
72°C	1 min	
60°C	60 min	
4°C	forever	

- Data analysis: 3730, 3130xl Genetic Analyzer

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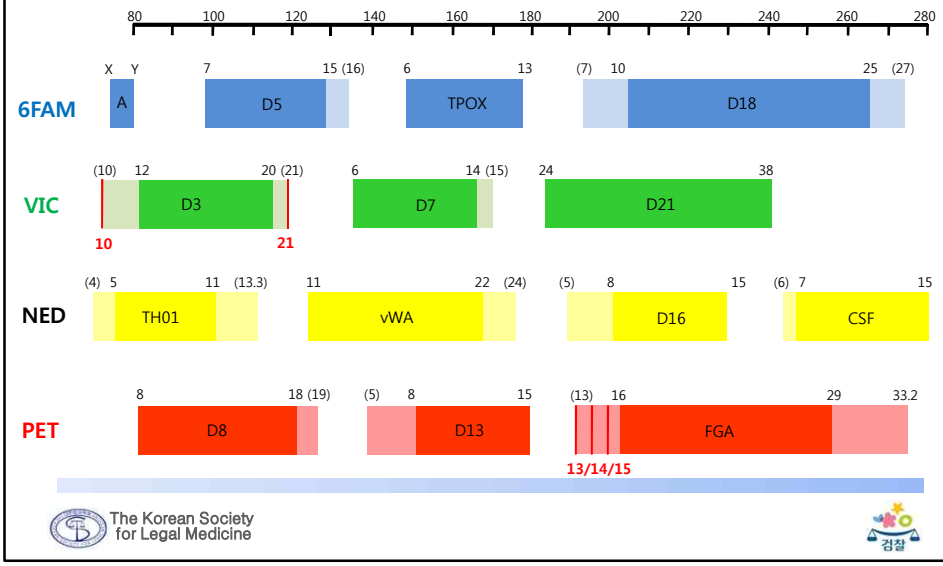
Kplex-14 System of Allelic Size Range



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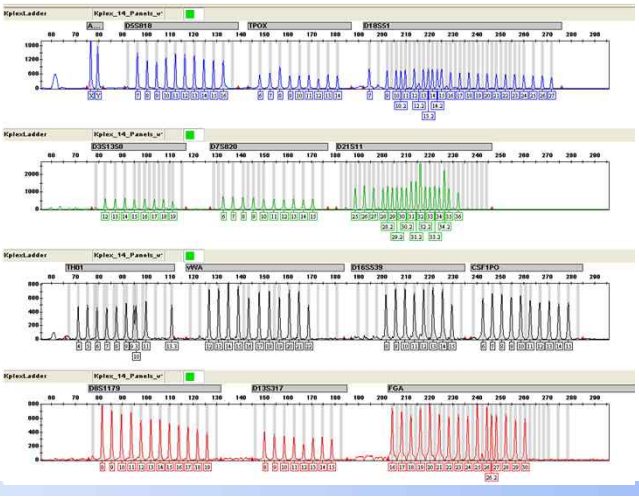
Comparison of Allelic Size Range

Locus	Allelic range	Amplicon size range (bp)		
		Kplex 14	Identifiler®	PowerPlex® 16
Amelogenin	X, Y	**77, 80**	107, 113	106, 112
D5S818	7-16	**97-133**	134-172 (+39)	119-155 (+22)
TPOX	6-13	**149-177**	222-250 (+73)	262-290 (+113)
D18S51	7-27	**193-273**	262-345 (+72)	286-366 (+93)
D3S1358	11-20	**82-118**	108-144 (+26)	111-147 (+29)
D7S820	6-15	**134-170**	255-291 (+121)	215-251 (+81)
D21S11	24-38	**184-240**	185-240 (0)	203-259 (+19)
TH01	4-13.3	**72-111**	163-202 (+91)	156-195 (+84)
vWA	11-24	**124-176**	155-207 (+31)	127-179 (+3)
D16S539	5-15	**189-229**	252-293 (+64)	264-304 (+75)
CSF1PO	6-15	**244-280**	305-342 (+62)	321-357 (+77)
D8S1179	8-19	**82-126**	123-170 (+44)	207-251 (+125)
D13S317	5-15	**143-183**	205-245 (+62)	168-208 (+25)
FGA	16-33.2	**204-274**	211-283 (+9)	322-390 (+116)

Kplex-14 System Allelic Ladder



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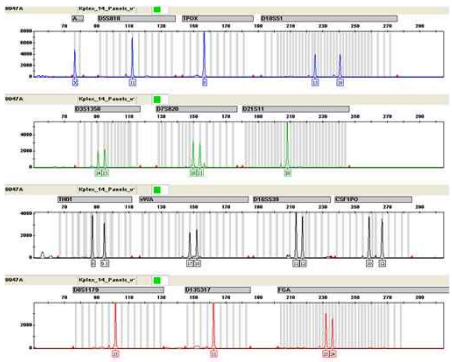
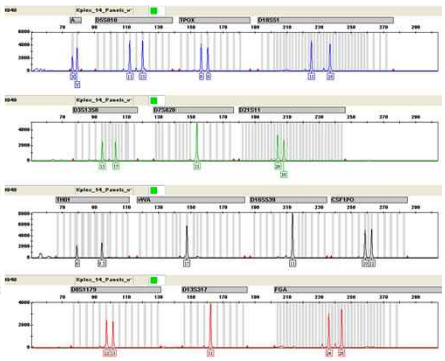
Profiles of Control DNA

9947A



9948



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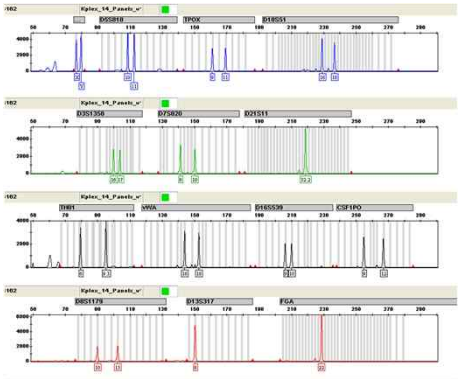
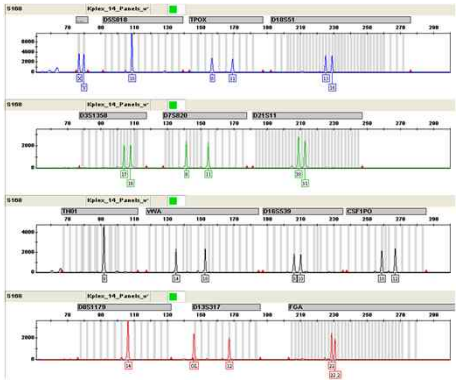
Profiles of DNAs

Sample 1



Sample 2



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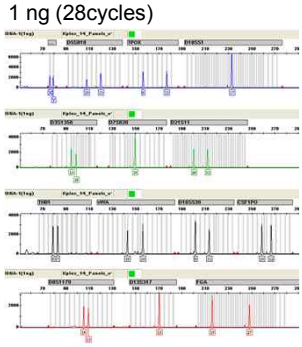
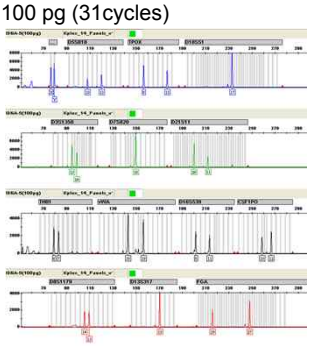
Sensitivity Test

- Using standard DNA in Quantifiler^{TM} kit

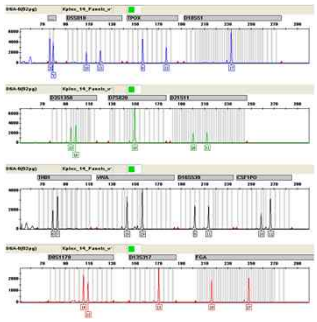
1 ng (28cycles)



100 pg (31cycles)



62 pg (31cycles)



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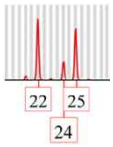
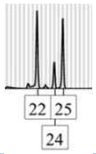
Concordance Test

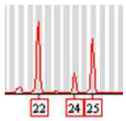
- 233개 시료의 유전자형 일치 (Kplex-14 vs. Identifiler®)

- 특이적 유전자형 비교

Loci	Identifiler®	PowerPlexHS16	Kplex-14
D18S51	Hetero + weak 3rd allele	Hetero + weak 3rd allele	Hetero + weak 3rd allele
vWA	Hetero + weak 3rd allele	Hetero + weak 3rd allele	Hetero + weak 3rd allele
FGA	Hetero + weak 3rd allele	Hetero + weak 3rd allele	Hetero + weak 3rd allele







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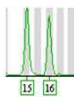
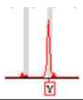
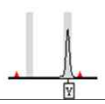
Concordance Test

Loci	Identifiler®	PowerPlexHS16	Kplex-14
D3S1358	Hetero (one allele weak)	Hetero	Hetero
CSF1PO	Homo	Hetero	Hetero
Amelogenin	YY	YY	XY







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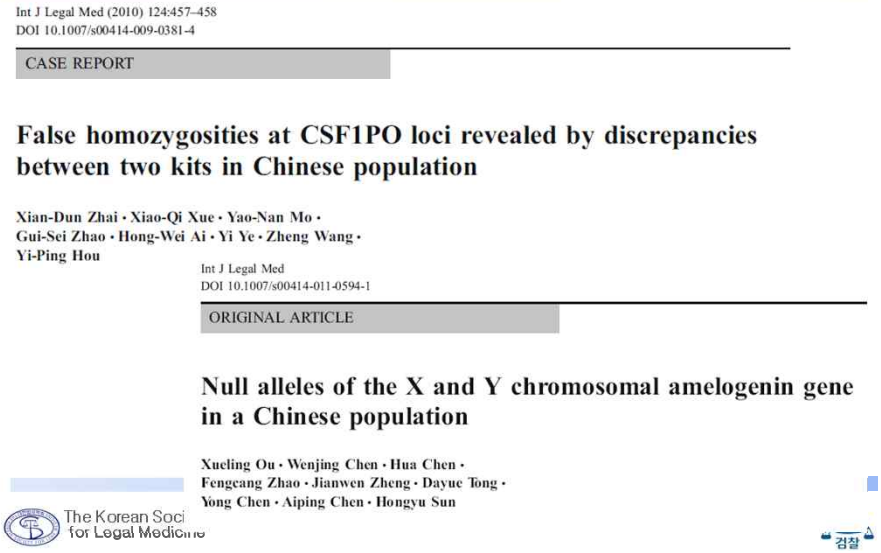
Concordance Test



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CASE REPORT

False homozygosities at CSF1PO loci revealed by discrepancies between two kits in Chinese population

Xian-Dun Zhai · Xiao-Qi Xue · Yao-Nan Mo · Gui-Sei Zhao · Hong-Wei Ai · Yi Ye · Zheng Wang · Yi-Ping Hou

Int J Legal Med
DOI 10.1007/s00414-011-0594-1

ORIGINAL ARTICLE

Null alleles of the X and Y chromosomal amelogenin gene in a Chinese population

Xueling Ou · Wenjing Chen · Hua Chen · Fengcang Zhao · Jianwen Zheng · Dayue Tong · Yong Chen · Aiping Chen · Hongyu Sun

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검찰

Summary

- Kplex-14 is constructed considering the rarely observed allele in Korean population
- Kplex-14 can be used for degraded DNA as well as reference samples, since it can amplify STR loci as smaller amplicon
- Kplex-14 can stably amplify small quantity of DNA, such as 100 pg
- Kplex-14 System is expected to save costs, especially when dealing with lots of samples
- Kplex-14 System is useful as an alternative replacing or supplementary kits

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Further Study

- Reagent change (i.e., PCR buffer, Taq polymerase) with self developed reagents
- Direct PCR will be applied to Kplex-14 System for reduction of analyzing time and cost