



# Development of the Kplex-23 Multiplex PCR System to Analyze 23 Forensic Markers

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## Introduction

In this era of globalization, the exchange of forensic data is vital to international investigations, and the use of forensic DNA typing kits that are compatible with the global DNA databases (e.g. databases produced by EU Prüm DNA Data Exchange Network and databases using the CODIS) is important to enhance the opportunity for successful international cross-border DNA comparisons. Therefore, we developed a new multiplex PCR system, the Kplex-23 system, which amplifies a total of 23 forensic markers in a single reaction. The Kplex-23 system kit contains Amelogenin, D1S1656, D2S441, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, CSF1PO, FGA, TH01, TPOX, vWA, DYS391 and Y-M175. These markers include STRs in the CODIS and the Extended European Standard Set as well as 2 commonly used STRs (D2S1338 and D19S433) in addition to amelogenin and 2 Y chromosomal markers. Following the multiplex PCR system construction, we carried out concordance study using 100 individual samples and sensitivity test using serially diluted DNA.

## Materials and Methods

### Forensic marker information and primer design

We collected the information of 23 forensic markers 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 Electrophoresis

An in-house multiplex PCR system was developed for the amplification of 23 forensic markers. The multiplex PCR system included the CODIS STRs, the Extended European Standard Set, D2S1338, D19S433 and Sex-typing markers. PCR amplifications were conducted in a final volume of 10 µl containing 1.0 ng template DNA, 1.0 µl of Gold ST<sup>®</sup>R 10X buffer (Promega), 4.0 U of AmpliTaq Gold<sup>®</sup> DNA polymerase (Applied Biosystems) and the proper concentration of primers.

Thermal cycling was performed under the following conditions : 95°C for 11 m; 29 cycles of 94°C for 20 s, 59°C for 90 s, 72°C for 60 s; and a final extension of 60°C for 45 min. Capillary electrophoresis was performed on automatic DNA sequencer, 3130xl Genetic Analyzer (Applied Biosystems).

### Concordance study

We analyzed 100 Korean samples using both the Kplex-23 system and the PowerPlex<sup>®</sup> Fusion system for confirming concordance of allele genotype.

### Sensitivity test

We conducted sensitivity test using serial diluted DNA (500 pg, 250 pg, 125 pg, 100 pg, 62.5 pg and 31.2 pg). An allele was scored when its peak height was above than 100 RFU.

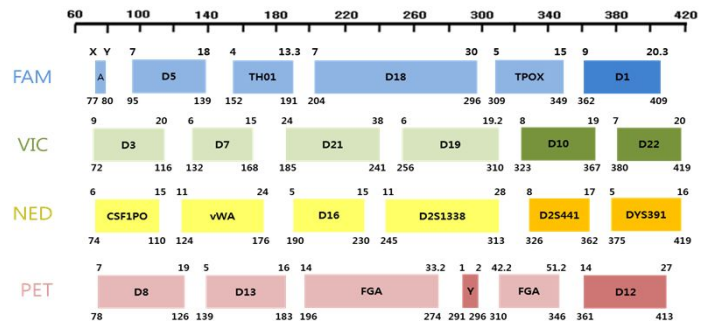
## Results

### 23 forensic markers of an in-house multiplex PCR system

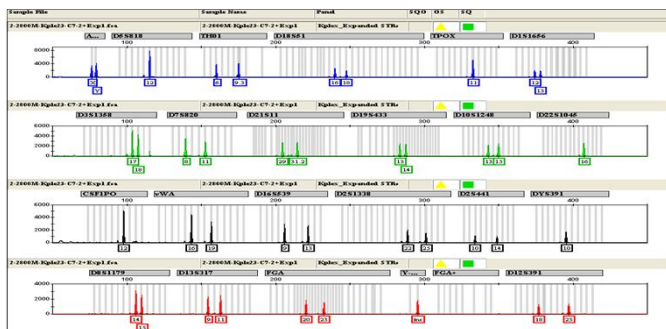
13 CODIS STR	Extended European Standard Set	Commonly used STR	Sex-typing marker
D3S1358	D1S1656	D2S1338	Amelogenin
D5S818	D2S441	D19S433	DYS391
D7S820	D10S1248		Y-M175
D8S1179	D12S391		
D13S317	D22S1045		
D16S539			
D18S51			
D21S11			
CSF1PO			
FGA			
TH01			
TPOX			
vWA			

Amelogenin  
+ 13 CODIS STRs  
+ 5 Extended European Standard Set  
+ 2 Commonly used STRs  
+ 2 Y-chromosomal markers  
= **Kplex system for 23 markers**

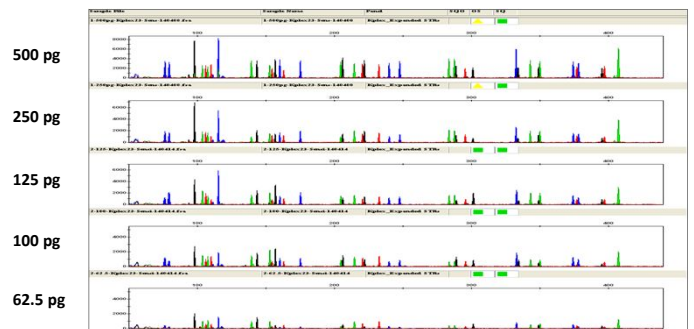
### Allelic size range for 23 loci of the Kplex-23 system



### Genotyping result of 2800M standard DNA using the Kplex-23 system



### Electropherogram of sensitivity test using serially diluted DNA



## Conclusions

The Kplex-23 system was developed to amplify common forensic markers of newly developed commercial analyzing kit, PowerPlex<sup>®</sup> Fusion (Promega) and GlobalFiler<sup>™</sup> (Life Technologies) systems. Primers were designed to produce amplicons with a size range of 70 bp to 420 bp detected by four fluorescent dyes. The Kplex-23 system has high sensitivity to provide successful genotyping results at all loci using 100 pg of genomic DNA without any allele drop-in or drop-out. In addition, concordance study in 100 Korean samples showed consistent results between the Kplex-23 system and the PowerPlex<sup>®</sup> Fusion kit. Multicenter validation study and development of direct amplification system for blood spots or buccal swab samples are also planned. In the future, the Kplex-23 system will be a useful tool for forensic casework and DNA database construction and it will enable fruitful international collaboration of criminal DNA information.

## Acknowledgement

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