



## Forensic validation studies on the Y-Plex™ 6 Kit

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### Abstract

The results of some forensic validation studies on the Y-PLEX™ 6 Kit (Reliagene Technologies) are presented. The evaluation of specificity (including imbalanced male/female mixtures, DNA from microorganisms) and sensitivity (28–32 cycles) allowed us to conclude that the Y-Plex 6 Kit system is a sensitive and reproducible Y-STR multiplex system. However, some nonspecific PCR products amplified from female DNA were observed for the yellow channel.

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### 1. Introduction

The Y-PLEX™ 6 Kit (Reliagene Technologies) is a commercial multiplex PCR system for the simultaneous analysis of six tetranucleotide STR loci (DYS393, DYS19, DYS389II, DYS390, DYS391 and DYS385) with high potential interest in some forensic DNA typing applications [1,2]. In this study, we present the results of some forensic validation studies including the following aspects: (1) evaluation of stutter bands, interlocus balance and precision study; (2) specificity studies for different amounts of female DNA and analysis of male/female mixtures; (3) sensitivity studies;

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Table 1  
Analysis of stutter bands

Marker	<i>N</i>	Mean (%)	Min (%)	Max (%)
DYS393	29	7.021	5.4	9.2
DYS19	25	6.038	4.1	9.7
DYS389	29	12.508	9.1	15.5
DYS390	18	8.203	5.3	10.2
DYS391	28	6.122	3.0	10.2
DYS385	20	3.513	4.3	10.1

(4) challenge microbial study against 32 DNA samples from different microorganisms; and (5) analysis of forensic cases previously analyzed with other autosomal and Y-STR systems including mixed samples from rape cases and bone samples from personal identification.

## 2. Evaluation of stutter bands, interlocus balance and precision study

### 2.1. Stutter bands and interlocus balance

Analysis of stutter bands was calculated as the peak height of stutter/peak height of the true allele of each locus (%). No significant differences with respect to the DNA input were observed for the range 1–20 ng. (Table 1, Fig. 1).

### 2.2. Precision study

Precision study was carried out by determining the size of the alleles of the allelic ladder run across eight different gels. The range of each allele is the difference between maximum and minimum size in base pair. Range is <0.5 bp for *DYS393*, *DYS19*, *DYS390* and *DYS391*; only for certain alleles of *DYS389* and *DYS385* the range is >1 bp.

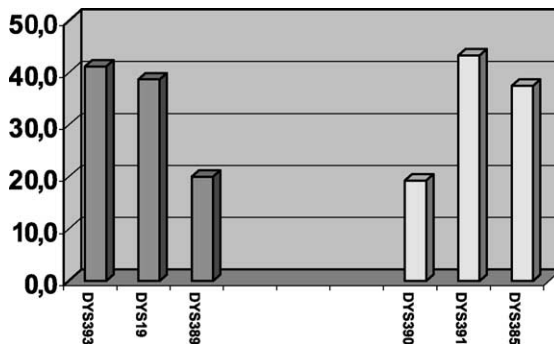


Fig. 1. Interlocus balance: 1 ng male + 12 ng female, 1 ng male + 120 ng female.

### 3. Specificity studies

#### 3.1. Female DNA

We have investigated the specificity of the kit against female DNA by typing different amounts of females DNA as well as imbalanced male/female mixtures of low amounts of male DNA. When the input of female DNA was higher than 10 ng, the following nonspecific PCR products were observed in the yellow channel. Fragments with high fluorescent intensity: 257 bp (in the range of *DYS391* marker) and 450 bp; fragments with low or medium fluorescent intensity: in the range of 215–300 bp (Fig. 2).

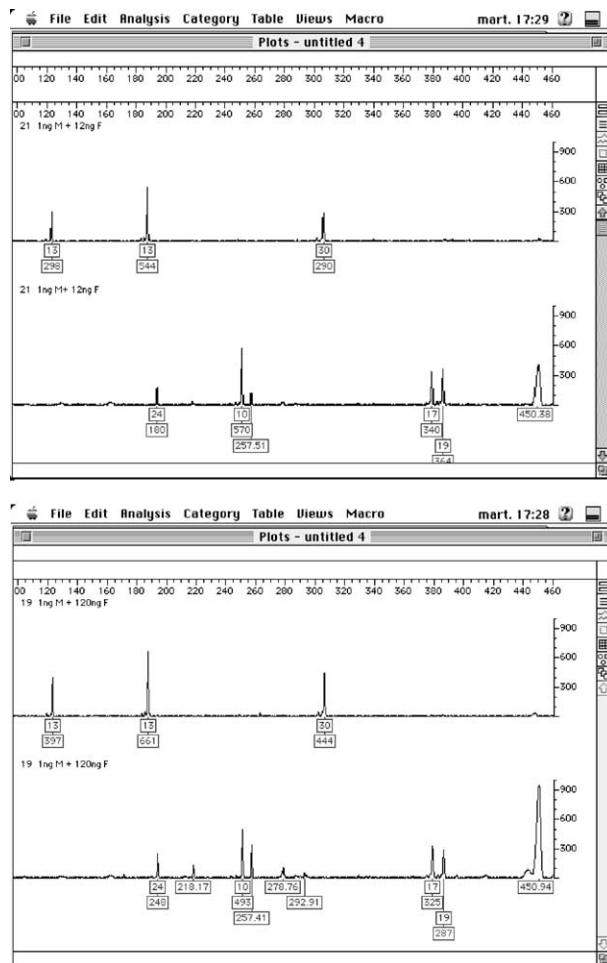


Fig. 2. Imbalanced male/female mixtures.

### 3.2. Microbial DNA challenge study

Chromosomal DNA samples from 32 different microorganism were analyzed: *Alcaligenes faecalis*, *Morganella morganii*, *Proteus mirabilis*, *Providencia stuartii*, *Shigella Sonnei*, *Acinetobacter calcoaceticus*, *Candida glabrata*, *C. krusei*, *Cryptococcus neoformans*, *Lactobacillus acidophilus*, *Rhodotorula glutinis*, *Streptococcus pneumoniae*, *S. boydii*, *S. flexneri*, *Trichosporon beigelii*, *C. guillermundii*, *Bacillus subtilis*, *Citrobacter freundii*, *B. cereus*, *Clostridium perfringens*, *Escherichia coli*, *Pseudomonas aeruginosa*, *P. stutzeri*, *Staphylococcus aureus*, *S. epidermidis*, *S. sanguis*, *Salmonella enteritidis*, *Micrococcus luteus*, *Vibrio alginolyticus*, *Corynebacterium* sp., *C. albicans* and *C. tropicalis*.

All microbial DNA failed to yield any detectable amplification products.

## 4. Sensitivity studies

Robust and reproducible amplification results for all the six loci were obtained for a DNA template input of 500 pg (28 cycles) or 250 pg (30 cycles). Although higher sensitivity was obtained with 32 cycles (100 pg), some nonspecific products were also observed for the yellow channel.

## 5. Analysis of forensic cases

Y-PLEX was also successfully amplified from 13 imbalanced semen/vaginal mixtures corresponding to five rape cases that could not be solved by autosomal STR profiling. All the typing results were consistent with those previously obtained in the analysis of two multiplex Y-STR systems developed in our lab [3].

We have also applied this Y-STR multiplex amplification system to the analysis of degraded DNA extracted from bone samples in two skeletal remains identification cases.

## 6. Conclusion

In conclusion, our data indicate that the Y-PLEX™ 6 Kit system yielded sensitive (up to 100 pg of male DNA, 32 cycles), reproducible and balanced typing results. However, some nonspecific PCR products amplified from female DNA were observed for the yellow channel. This lack of specificity could be a drawback for some casework applications.

## References

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