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Validation of the Y-Plex 6™ kit

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Abstract. With the aim of using Y-chromosomal polymorphic markers in Swiss crime cases and deficiency paternity cases, a validation study of the Y-Plex 6™ kit (Reliagene) was performed. The Y-Plex 6 kit is a commercial multiplex system for the simultaneous analysis of six tetranucleotide STR loci (DYS393, DYS19, DYS389II, DYS390, DYS391 and DYS385). In this study, we present the results of some forensic validation studies including the following aspects: sensitivity, influence of the increase of number of cycles (28–34 cycles), evaluation of stutter peaks, specificity study for different amounts of female DNA, analysis of female/male mixtures, analysis of male/male mixtures, analysis of father–son pairs, a forensic case application and quality control.

Keywords: Y-STR; Validation study; Forensic analysis

1. Introduction

Y-chromosomal STR polymorphisms are of increasing interest in the forensic field because of their possible application in stain analyses and in deficiency paternity cases. Since the majority of sexual offenses have a male perpetrator and a female victim, using Y-chromosome specific primers can improve the chances of being able to detect small amounts of perpetrator DNA in a high background of heterologous female DNA. We used the commercial kit Y-Plex 6™ developed by Reliagene. The PCR was carried out following the amplification conditions recommended by the manufacturer. Fragments were analyzed on an ABI310 instrument.

2. Sensitivity

Robust and reproducible amplification results for all six loci were obtained for a DNA template input of 150 pg (30 cycles) (Fig. 1). Increasing the number of cycles (32–34) made it possible to increase the sensitivity to amounts of < 150 pg.

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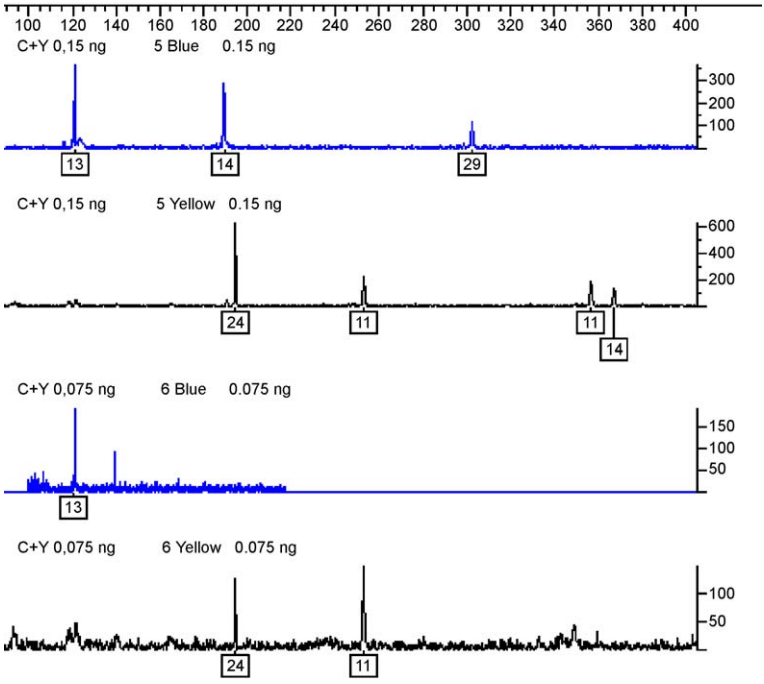

Fig. 1. 0.15 and 0.075 ng at 30 cycles.

3. Stutter peaks

Analysis of stutter peaks was calculated as the peak height of the stutter/peak height of the true allele of each locus (%). The stutter percentage is dependant on the allele length, the larger fragments having the highest stutter percentage. The locus DYS389II has the highest stutter percentage of all six loci, between 10% and 13%; this may complicate the interpretation of mixtures of male DNA.

4. Specificity: female DNA

Female DNA in concentrations ranging from 2 to 50 ng were amplified. When the input of female DNA was higher than 2 ng, two nonspecific peaks using the TAMRA dye (yellow) were observed. The first was at 255.8 bp. This peak was in the range of the DYS391 marker but not in an allelic window. A second constant nonspecific peak was observed at 450 bp, clearly outside the range of the DYS385 locus.

5. Specificity: mixture male/female DNA

The male/female mixtures were prepared in the proportions 1:0, 1:1, 1:5 and 1:20. A complete male profile was detected in male/female mixture samples down to the 1:20 ratio, which contained 1 ng of male DNA and 20 ng of female DNA.

6. Selectivity: mixture male/male DNA

The male/male mixtures were prepared in the proportions 1:0, 19:1, 9:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:9, 1:19 and 0:1. Male No. 2 showed not one but two alleles at the DYS389II locus (30 and 32), due probably to a duplication of the locus. The results indicated that the quantities of amplified products are generally proportional to the DNA present in the mixture. The complete DNA profile of male 2 was detected in mixtures up to 1:4 (male No. 2: male No. 1). An incomplete DNA profile of male 2 was detected in a mixture of 1:9 (male No. 2/male No. 1).

7. Father–son pairs

Twenty-five Swiss father–son pairs were typed; no inconsistencies were found. The father–son pairs had previously been investigated for 15 autosomal STRs, with paternity indices exceeding 100 000.

8. Swiss population data

Swiss population data have already been published [1,2].

9. Forensic case application

As an example of the application of Y-STRs, a case study is provided. Two blood stains found at a crime scene were typed using the SGM Plus kit. The two profiles were similar; it was suspected that the two men who left the blood stains at the crime scene were brothers. To investigate this theory, the DNA extracts of the crime scene material were typed with the Y-Plex 6™ kit. The two haplotypes were identical and when compared with the Y haplotype reference database (Berlin), a frequency of 0.00028 was calculated. Combining the autosomal data and the Y-STR data, it is extremely likely that the two men who left the blood stains at the crime scene are from the same paternal lineage (i.e. brothers).

10. Quality control

The Y-Plex 6™ kit was successfully used in the following proficiency testing: German proficiency test (GEDNAP 24 and 25), ISFG paternity testing 2003 and a Swiss quality control organized by the Swiss Society of Legal Medicine.

11. Conclusions

Our data indicate that the Y-Plex 6™ kit yielded sensitive (up to 150 pg of male DNA, 30 cycles), reproducible and reliable typing results for forensic casework and deficiency paternity testing.

References

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