



Evaluation of the 12 Y-STR loci in the PowerPlex® Y-system; experience from analyses of single male samples and simple male–male mixtures

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Abstract. As part of an in-house evaluation of the PowerPlex® Y-system, we have measured sensitivity and male specificity as well as the proportion of stutter bands and spectral bleed-through in the 12 Y-STR loci included in the kit. The results from the validation of the PowerPlex® Y-system are in good concordance with other validation studies except the low amplification efficiency of locus DYS393 observed in the present study. Results from analyses of male–male mixtures of males with known Y-haplotypes indicate that in simple mixtures (two males) with minor contributor constituting between 10% and 60% of the major contributor, the peak area of unshared alleles provides quantitative information that might be used to interpret the two most likely haplotypes. If the minor contributor constitutes less than 10% of the total sample, a possible drop-out of minor component must be taken into consideration when interpreting the “minor haplotype.” © 2005 Elsevier B.V. All rights reserved.

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1. Introduction

Interpretation of sample mixtures require knowledge about the efficiency of the multiplex system. As part of an in-house evaluation of the PowerPlex® Y-system, we have measured sensitivity and male specificity as well as the proportion of stutter bands and spectral bleed-through (pull-up) in the 12 Y-STR loci included in the kit. In addition, we have analysed male–male mixtures with different proportions of DNA from two contributors. Based on these results, we have evaluated the use of quantitative information (peak area) in interpretation of the most likely haplotypes in simple mixtures.

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2. Materials and methods

Direct amplification was performed from samples on FTA[®] paper (Whatman, Clifton, NJ, USA). The Chelex method [1] was used for DNA extraction of all other samples. Samples were quantified using QuantiBlot[®] Human DNA quantitation kit (Applied Biosystems, Foster City, CA, USA) [2]. Y-STRs were PCR amplified using the PowerPlex[®] Y-system and PCR products were separated by capillary electrophoresis using an ABI PRISM[®] 3100 as described by the manufacturer. Genotyping was performed with the allelic ladder provided with the kit and Genotyper software[®] (Applied Biosystems) with the PowerTyper[™] Y Macro (Promega Corporation). The minimum interpretation threshold used was 50 relative fluorescent units (rfu). The proportion of stutter band was calculated by dividing the peak area (rfu) of stutter band by the peak area (rfu) of the true allele and multiplying by 100. Calculations were only performed at loci with observable stutter bands and when true allele was ≥ 6000 rfu the stutter band was not included (to avoid data anomalies created by signal saturation). The proportion of pull-up of another colour under the main peak was calculated by dividing the peak area (rfu) of pull-up peak by the peak area (rfu) of the true allele and multiplying by 100.

Combinations of three samples from males with known haplotypes were used to compose male–male mixtures with 5-, 10- and 20-fold excess of the major contributor. A total of nine samples were analysed.

Combinations of 14 samples from males with known Y-haplotypes were used to compose male–male mixtures with mixture ratios within the interval 1:3 to 1:1. A total of 65 samples were analysed. Disregarding DYS393 and DYS385a/b the peak areas of alleles in each sample were used to type a “minor” and a “major” Y-haplotype consisting of all minor alleles or major alleles, respectively, in loci with unshared alleles (Fig. 1).

3. Results

The sensitivity of the kit was evaluated by titration of male DNA (three different male samples). Full profiles was obtained in all samples with ≥ 0.2 ng template. Balance between loci was good except locus DYS393 which often showed low peak area values ($\leq 60\%$) compared to the other loci. As a consequence of this, DYS393 was the most commonly observed locus to drop-out at low template concentrations. Male specificity was tested in mixtures with male (0.5 ng) to female ratios of 1:1 to 1:20. All samples showed full male profiles and with no noticeable decrease in peak heights with increasing amount of female DNA.

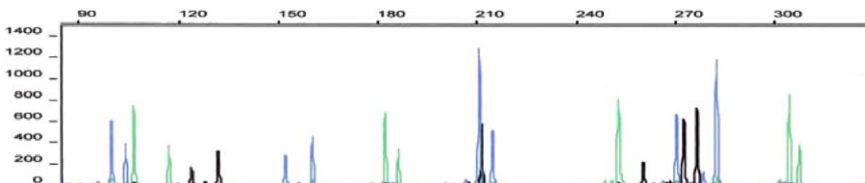


Fig. 1. Electropherogram showing Y-STR results from typing of a male–male mixture. In this sample the peak areas of minor contributor's alleles are less than 60% of major contributor's alleles in loci with unshared alleles. Two Y-haplotypes were typed using the quantitative information from peak areas: (a) Major haplotype: major allele in two-allele loci combined with shared allele in loci with one allele. (b) Minor haplotype: minor allele in two-allele loci combined with shared allele in loci with one allele.

Table 1
Average stutter values for the Y-STR loci in the PowerPlex® Y-system

	DYS391	DYS389I	DYS439	DYS389II	DYS438	DYS437	DYS19	DYS392	DYS393	DYS390	DYS385a/b
Stutter ^a	5.6%	5.4%	6.2%	11.2%	3.0%	5.3%	5.8%	8.24%	7.3%	8.0%	9.1%
S.D. ^b	1.06	0.99	1.55	2.14	0.90	1.51	1.25	2.16	1.61	1.54	3.12
<i>n</i> ^c	35	21	35	46	13	18	24	35	19	19	45
Repeat type	Tetra	Tetra	Tetra	Tetra	Penta	Tetra	Tetra	Tri	Tetra	Tetra	Tetra
Allele range ^d	6–14	9–17	9–14	24–34	6–14	13–17	10–19	6–17	9–17	17–28	7–28

^a Average stutter band values.

^b One standard deviation.

^c Number of samples with stutter.

^d Allele range with allele sizes given as number of repeats.

Proportion of stutter bands was measured in 50 male samples (Table 1). Tetranucleotide loci with large alleles (e.g., DYS389II) showed increased proportion of stutter bands compared to tetranucleotide loci with small alleles (e.g., DYS389I). The trinucleotide locus DYS392 showed a slightly increased proportion of stutter band compared to other loci with similar allele ranges. The pentanucleotide locus DYS 438 was the best performing locus with the lowest proportion of stutter band. The proportion of stutter bands was less than 20% for all loci tested. Stutter bands in position $N-2$ (DYS389I/II, DYS385 a/b) and $N+1$ (DYS392, DYS439, DYS391) was observed, but always at much lower proportions than stutter bands in position $N-1$. At locus DYS19, a peak in position -2 bp was observed in addition to the stutter band at $N-1$ (-4 bp).

No pull-up peaks larger than 10% was observed in the samples analysed ($n=30$). Most frequent pull-up peaks observed were green peaks in a main peak of blue.

Drop-out of minor component in male–male mixtures was observed when it constituted less than 10% of the total sample. In mixtures with minor component (0.5 ng template DNA) constituting less than 5% of the total sample, the minor component usually dropped out. A “major haplotype” and a “minor haplotype” (Materials and methods, Fig. 1) was typed in 65 male–male mixture samples (disregarding locus DYS393 and DYS385 a/b). The major and minor haplotype corresponded to the two correct haplotypes in all mixture samples ($n=42$) where relative peak area difference of unshared alleles (average peak area of minor alleles divided by average peak area of major alleles) was ≤ 0.6 .

4. Discussion

The results from the mixture analysis indicate that in simple mixtures (two males) with minor contributor constituting between 10% and 60% of the major contributor, the peak area of alleles provides quantitative information that might be used to interpret the two most likely haplotypes. If the minor contributor constitutes less than 10% of the total sample, a possible drop-out of minor component in loci with one allele only must be taken into consideration when interpreting the “minor haplotype.” The results from the validation of the Powerplex® Y-system are in good concordance with other validation studies [3] except the low amplification efficiency of locus DYS393 observed in the present study. Suboptimal ramping conditions in the PCR amplification step might be a possible explanation for this low efficiency of DYS393 PCR-amplification.

References

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