

## Study to compare three commercial Y-STR testing kits

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**Abstract.** An evaluation study was carried out to test the performance of three commercially available Y-STR DNA profiling kits for their suitability to forensic case work. The three kits assessed were Reliagene's Y-Plex™ 12 kit, Promega's PowerPlex® Y system and Applied Biosystems' AmpFISTR® Yfiler™ kit. Four experiments were devised to assess the performance of the three kits. Allelic peak height data was used to measure the reproducibility, sensitivity, male specificity and ability to discriminate male mixtures of the three kits. Samples were processed following the manufacturers recommended protocols. PCR products were run on 3100 electrophoresis platforms and the resultant DNA profiles analysed using GeneScan and Genotyper analysis software packages. All three kits gave reproducible results with concordant genotypes between replicates and kits. Average peak height data showed the AmpFISTR® Yfiler™ kit to be the most reproducible kit during the evaluation study. PowerPlex® Y system was shown to be the most sensitive kit during the evaluation study. All three kits gave full male profiles for all samples processed in the specificity experiment. There was no evidence of female artefacts in the PowerPlex® Y and AmpFISTR® Yfiler™ samples; however, there was evidence of additional female artefacts in all Y-Plex™ 12 samples. The AmpFISTR® Yfiler™ kit showed the least degree of variation in peak area ratio's for the expected male mixture ratio's and therefore showed that it was able to discriminate male mixtures better than the PowerPlex® Y and Y-Plex™ 12 kits during this evaluation study. © 2006 Published by Elsevier B.V.

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### 1. Introduction

Y chromosome specific short tandem repeat (Y-STR) markers are becoming popular tools to identify males in forensic situations. Because the Y-STRs reside on male specific

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DNA, female DNA is not reactive. Consequently, Y-STRs can be exploited for the use in analysis and interpretation of male/female mixtures often encountered in casework. In cases of sexual assaults with a male perpetrator and a female victim, Y-STRs can improve the chances of detecting low levels of the perpetrators DNA in a high background of the female victim's DNA. This paper details an evaluation study to test the performance of three commercially available Y-STR profiling kits for their suitability to forensic case work.

## 2. Scope of study

The following Y-STR kits were included in this study:

- Reliagene Y-Plex™ 12 kit—11 STRs
- Promega Poweplex® Y system—12 STRs
- Applied Biosystems AmpFISTR® Yfiler™ kit—17 STRs

All three kits included all loci included in the European minimal haplotype and those recommended by SWGDAM.

## 3. Reproducibility

Five male samples were amplified in duplicate for each of the three kits. All three kits gave full balanced profiles with comparable results seen between duplicate samples. There was also complete concordance between the common loci of the three kits.

## 4. Sensitivity

A DNA dilution series ranging from 2 ng to 31.25 pg was used to test the sensitivity of the three kits to levels of DNA in the PCR reaction. The PowerPlex Y and the Y-Plex 12 kits were shown to be more sensitive than the Y filer kit. Calculations of the percentage drop out of alleles with 31.25 pg of DNA added, showed the PowerPlex Y kit to be slightly more sensitive than the Y-Plex 12 kit (Table 1).

Table 1  
Profile scores given to the samples processed during the sensitivity experiment

Kit	Sample	31.25 pg	62.5 pg	125 pg	250 pg	500 pg	1 ng	2 ng
Reliagene Y-Plex™ 12	A	PP (-6)	PP (-1)	FP	FP	FP	FP	FP
	B	PP (-5)	PP (-3)	FP	FP	FP	FP	FP
PromegaPoweplex® Y	A	PP (-5)	PP (-1)	FP	FP	FP	FP	FP
	B	PP (-7)	PP (-1)	FP	FP	FP	FP	FP
Applied Biosystems AmpFISTR® Yfiler™	A	PP (-11)	PP (-7)	FP	FP	FP	FP	FP
	B	PP (-11)	PP (-6)	PP (-2)	FP	FP	FP	FP

Key: PP – partial profile, FP – full profile. The number in brackets shows how many peaks were missing.

## **5. Specificity**

Male and female DNA samples were mixed together. 0.5 ng of male DNA was added to each reaction, while the amount of female DNA added was varied to create the following ratios of male to female DNA: 1:125, 1:250, 1:500 and 1:1000.

All three kits gave full male profiles for all samples processed. There was no evidence of 'female' artefacts in the PowerPlex Y and Yfiler samples however there was evidence of additional 'female' artefacts in all Y-Plex 12 samples. Large, non-allelic artefacts were seen in the same positions for all the ratios tested but did not increase in height as the amount of female DNA increased. It would appear that 75 ng of female DNA was sufficient to create off scale artefacts (greater than 4000 RFU) in the profiles. This suggests that the Y-Plex 12 kit may not be completely specific for male DNA.

## **6. Discrimination of male mixtures**

Two male DNA samples were prepared in the following mixture ratios 1:1, 3:1, 10:1 and 20:1. Allelic peak heights in each mixture were then compared.

All three kits gave full profiles for the major male sample. For the Y-Plex 12 and PowerPlex Y kits, full profiles for the minor male sample were obtained in three out of the four mixture ratios, while full profiles were obtained for the minor male for all four mixture ratios with the Yfiler kit.

## **7. Conclusions**

All three kits tested performed well in the study with similar sensitivities, reproducibility and ability to distinguish mixtures.

Reliagene Y-Plex™ 12 kit showed evidence of artefact peaks when excess female DNA was present, possibly indicating reduced specificity compared to the other two kits.

The Promega Poweplex® Y kit was shown to be slightly more sensitive than the Reliagene Y-Plex™ 12 kit and quite a lot more sensitive than the Applied Biosystems AmpFISTR® Yfiler™ kit. So the Promega Poweplex® Y kit may work better with degraded DNA samples.

The Applied Biosystems AmpFISTR® Yfiler™ kit types 17 STRs as oppose to 12 STRs typed by each of the other two kits. Since the Y chromosome is passed down from father to son without any recombination, it is not as variable between individuals as other STRs and results from individual markers cannot be combined using the product rule. Therefore, having more markers is an advantage for the Applied Biosystems AmpFISTR® Yfiler™ kit allowing a higher degree of discrimination to be possible.