Developmental validation of the Yfiler® Plus PCR amplification kit

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ABSTRACT

Y-chromosomal markers have proven useful in solving investigations where low levels of male DNA are recovered along with high levels of female DNA. An intrinsic limitation of Y-STRs compared to autosomal STRs is a reduced power of discrimination due to a lack of recombination throughout most of the Y-chromosome. Thus, in an effort to increase the power of discrimination we have developed a new 6-locus, 27plex Y-STR system that includes the 17 markers from the AmpFISTR Yfiler® and Yfiler Direct kits plus 10 additional highly polymorphic Y-STR markers (YF557n, YF560n, Y561, Y562, Y563, Y564, Y565, DY566, DY567a, DY568, DY569). These ten new loci include 7 rapidly mutating Y-STR loci which allow for improved discrimination of related individuals.

The new multiplex is a dual application assay designed to amplify DNA from extracted casework samples and database samples from storage cards and swab lysates via direct amplification. Compared to the previous Yfiler® and Yfiler® Direct kits, the new multiplex shows improved performance in inhibited samples and advanced female and male sample panels. Additionally, better differentiation between male-male mixture samples in high female DNA background, and faster time to results. Additionally, no reproducible cross-reactive products were obtained from bacteria or commonly encountered animal species. The haplotype diversity and discriminatory capacity calculations for several population groups will be presented, as well as further studies and validation studies demonstrating improved performance with challenging samples.

INTRODUCTION

The Yfiler® Plus Kit, a dual application assay designed for amplification of extracted DNA from casework samples and database samples from storage cards and swab lysates, shows improved performance over the Yfiler® Kit. The Yfiler® Plus Kit is a dual application kit designed for amplification of database samples YHRD.org and usystrdatabase.org, as well as Life Technologies internal population database. Yfiler® Plus Kit demonstrates robust performance with all direct kits plus 10 additional highly polymorphic Y-STR markers, 7 of which are rapidly mutating Y-STR loci. This allows for improved discrimination of related individuals. The data demonstrates optimal peak height, as well as good intracolor balances for all sample types tested.

RESULTS

Figure 3: Yfiler® Plus Kit Power of Discrimination

Table: Sensitivity Study: Alleles Recovered

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Figure 4: Yfiler® Plus Kit Allelic Ladder

Figure 5: Control DNA 007 amplification with Yfiler® Plus Kit

Figure 6: Mixture amplification with Yfiler® Plus Kit

Figure 7: Direct amplification of database samples with the Yfiler® Plus Kit

Figure 8: Inhibitor tolerance with Yfiler® Plus Kit

Figure 9: Yfiler® Plus Kit Direct Amplification Performance

CONCLUSION

The Yfiler® Plus Kit is a dual application assay designed for amplification of extracted DNA from casework samples as well as direct amplification of multiple database sample types (Blood and Buccal samples on treated paper, Blood samples on untreated paper with addition of Prep-n-Ga buffer, and Buccal swabs). The Yfiler® Plus Kit shows excellent performance over the Yfiler® Kit in amplification of both male and female mixture samples. The improved master mix formulation enables better tolerance to high levels of inhibitors, resulting in full profiles compared to no amplification with the Yfiler® Kit. PCR amplification time is <95 mins for a 30 cycle amplification protocol. The Yfiler® Plus Kit demonstrates robust performance with all direct amplification sample types tested, resulting in high first pass success rates.

REFERENCES


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