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The benefits and limitations of expanded Y-chromosome short tandem repeat (Y-STR) loci

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ABSTRACT

Recent advances in commercially available Y-chromosome short tandem repeat (Y-STR) profiling kits include the development of expanded locus sets and the inclusion of rapidly mutating (RM) Y-STR loci. Thermo Fisher Scientific recently released its Yfiler® Plus (YF+) kit to the forensic market. Yfiler® Plus has 27 loci, including 7 RM Y-STRs and full locus overlap with its predecessor AmpFlSTR® Yfiler™ (Yfiler). Forensic Science SA has been using Yfiler in routine forensic casework since 2007 but recently evaluated YF+ due to its apparent increased sensitivity and discrimination power. As with any transition to a new multiplex, there are benefits and limitations which need to be considered by each laboratory before implementation proceeds. Whilst there are a number of cost, profile interpretational and laboratory issues that need to be overcome, the benefits that YF+ provides over Yfiler will result in FSSA implementing this expanded multiplex for forensic casework.

1. Introduction

Y-chromosome short tandem repeat (Y-STR) analysis using the 17 locus AmpFlSTR® Yfiler™ kit (Thermo Fisher Scientific, Waltam, MA, USA) was introduced into casework at Forensic Science SA (FSSA) in 2007. This kit has been widely used in casework since that time and has provided probative information in many cases. Thermo Fisher Scientific recently released its 27 locus Yfiler® Plus (YF+) kit to the market. This kit has full overlap with the 17 loci contained in Yfiler™ with the addition of 10 new markers, 7 of which are rapidly mutating (RM) Y-STRs. FSSA has recently completed a within-laboratory verification of YF+ (Simon et al., in preparation) and compared its in-house performance to that of its predecessor. This verification was undertaken with the goal to implement YF+ in 2015.

With the implementation of any new multiplex, there are a number of benefits and limitations which require consideration by the laboratory. Whilst YF+ offers many improvements to routine forensic casework at FSSA, there are some issues associated with moving away from Yfiler to this expanded multiplex. The benefits and limitations of implementing YF+ at FSSA are herein discussed.

2. Benefits of implementing Yfiler® Plus

2.1. Increased sensitivity

Internal evaluation of YF+ showed that it had at least a 10 to 20-fold better sensitivity than Yfiler with alleles detected at just 1 pg of input DNA. As forensic DNA samples often contain less than optimal amounts of DNA, the improved sensitivity of YF+ ensures that the maximum allele information is generated for inclusionary and exclusionary purposes. The increased sensitivity may also enable low level contributors in mixed male samples to be detected and profiles to be generated for additional case situations where Yfiler has not proven successful at FSSA (*e.g.* digital rapes).

2.2. Discrimination and exclusionary power

The inclusion of 10 additional loci in YF+ has increased the overall haplotype diversity and discrimination power for different populations [1,2]. This increased haplotype diversity may result in lower match probabilities as matching Yfiler haplotypes may be

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resolved with the additional loci included in YF+. The increased amount of profile information generated from the additional loci will also be beneficial for exclusionary purposes, especially when dealing with low level profiles where allelic information may be missing.

2.3. Less inhibition

The intensity of the male profile generated using Yfiler is mildly inhibited by high background levels (1000-fold) of female DNA (FSSA internal validation report). YF+ does not demonstrate this same effect and is therefore more likely to detect low amounts of male DNA in a mixed sample containing excess female DNA. The performance of YF+ for non-probative casework samples affected by inhibitors and DNA degradation is also very high.

2.4. Improved mixture resolution

Due to the additional loci, YF+ is better able to resolve the correct number of contributors to a mixed profile than Yfiler as the probability of non-shared alleles is higher. The increased sensitivity also assists in the identification of low level contributors to a mixture.

2.5. Direct PCR

FSSA generates reference DNA profiles from samples stored on Whatman FTA® cards (GE Healthcare Life Sciences, NSW, Australia) in their standard workflow. YF+ gave a high first pass success rate (86%) for direct PCR of reference FTA® samples. FSSA's current reference sample protocol using Yfiler requires the FTA® discs to be washed prior to PCR to obtain a similar success rate. As this is not required for YF+, reference sample processing will be quicker and easier to automate.

2.6. Rapidly mutating Y-STRs

A major limitation of Y-STRs is that any profile matching an offender is also likely to match his paternal male relatives. Therefore, in the absence of supporting autosomal data, any non-exclusion is of limited value in a court setting. The addition of RM Y-STR markers [3,4] to YF+ has significantly improved discrimination between closely related males [3]. This will enable paternal male relatives to be eliminated from an investigation in some instances and effectively mitigate arguments, such as the Brother Defence, in a court situation.

2.7. Cost

When only casework sample analysis is considered, the implementation of YF+ at FSSA is cost neutral with the cost of analysis per sample being on par with Yfiler despite the expanded locus set. However, wider application of YF+ to routine casework as a result of its greater sensitivity and discrimination power may increase the number of tests performed annually.

3. Limitations of implementing Yfiler® Plus

3.1. Contamination

The increased level of sensitivity offered by YF+ increases the risk of detecting incidental contaminating DNA. FSSA experienced this issue after the implementation of GlobalFiler® (Thermo Fisher Scientific, Waltam, MA, USA) which also has increased sensitivity over previous generation autosomal STR multiplexes. Strategies, such as enhanced laboratory cleaning regimes, sourcing of certified DNA-free consumables, and effective checks for staff, intra- and inter-batch contamination events, will need to be implemented by FSSA to combat this increased risk of contamination.

3.2. New interpretational guidelines

New in-house guidelines for the interpretation of low level and mixed YF+ profiles need to be developed. These guidelines require generation and analysis of large amounts of empirical data to identify appropriate peak height ratios for safe separation of mixtures and stochastic thresholds where allelic drop-out is possible. In addition, the interpretational experience accumulated by FSSA's Reporting Officers for Yfiler will need to be re-established following transition to YF+.

3.3. Haplotype database/Cost

One main barrier to the implementation of YF+ is the time and cost (>$50K AUD) required to upgrade FSSA's existing regional haplotype databases as the Y-chromosome Haplotype Reference Database (https://yhrd.org) [5] does not contain YF+ data for Australian sub-populations. As the strength of any inclusionary statistic is based upon the size of the haplotype database to which the YF+ profile is being compared, FSSA plans to increase the size of the databases it currently uses. However, this incurs additional cost beyond that of the YF+ kit purchase as FSSA performs Y-SNP typing to correctly assign Y-chromosome ancestry.

3.4. Inclusionary probability

If the observed Yfiler haplotype is rare and has not been observed more than once in the relevant haplotype database, then expanding the profile to include YF+ loci is unlikely to increase the inclusionary probability for an individual. This is because the typing of additional loci is unlikely to change the overall haplotype frequency in the population.

3.5. Rapidly mutating Y-STRs

Whilst RM Y-STRs are useful to eliminate paternal male relatives from criminal investigations, they may prove disadvantageous for missing person and disaster victim identifications when matching haplotypes between paternal relatives are used to identify deceased persons. Requests for familial searches of investigative DNA databases to identify potential relatives of offenders are also increasing at FSSA. The inclusion of RM markers may also impact on these searches as Y haplotypes are used to further refine possible familial links between male individuals. Internal guidelines and Reporting Officer training for the interpretation of results obtained from RM Y-STRs will need to be developed to assist when haplotype differences are purely a result of mutation events.

4. Conclusion

FSSA has assessed the scientific validity and reliability of YF+ and will implement this kit later in the year due to the significant benefits it offers for routine casework. Whilst there are a number of cost, interpretational and laboratory issues that need to be overcome, these issues are not prohibitive to implementation. Overall, FSSA believes that the implementation of YF+ will result in an improved forensic science service and are currently working to

overcome the limitations addressed in this paper so its full benefit can be realised.

Conflict of interest

None.

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