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Evaluation of the Discriminatory Capacity in Commonly Used Y-STR Systems: Establishing a Reference for Forensic Interpretation

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Abstract

In this study, two commonly used Y-chromosome short tandem repeat (Y-STR) kits—the PowerPlex® Y23 System and the Yfiler™ Plus PCR Amplification Kit—were validated and subjected to statistical analyses. We compared the discriminatory capacity (DC) of the kits: AmpFLSTR™ Yfiler™ (17 loci), DC = 0.897 (N = 970); PowerPlex® Y23 (23 loci), DC = 0.978 (N = 418); Yfiler™ Plus (27 loci), DC ≈ 1 (N = 574); and for the 21 overlapping loci between PowerPlex® Y23 and Yfiler™ Plus, DC = 0.955 (N = 970). These results indicate that the Y-STR kits with more loci effectively enhanced the DC. We also observed that rapid-mutation Y-STR loci exhibited higher gene diversity (GD) and revealed no obvious differences between Taiwanese males and other groups. Because partial profiles are common in forensic DNA, we proposed a method for estimating the minimal number of Y-STR loci sufficient for interpretation, which forensic laboratories could use under their guidelines. Ordering loci by ascending GD, we sequentially selected the lowest 8~23 loci (for PPY23) and 8~27 loci (for YFP27) and recomputed the number of unique haplotypes (UH) and DC. Using the first 12 loci, PPY23 yielded 321 UH among 418 samples (DC = 0.768; match probability [MP] = 0.0048; haplotype diversity [HD] = 0.998), whereas YFP27 yielded 423 UH among 574 samples (DC = 0.737; MP = 0.0047; HD = 0.997). These benchmarks provide practical guidance for reporting and interpreting evidentiary Y-STR profiles.

Keywords: ***forensic science; Y-chromosomal short tandem repeat (Y-STR); discrimination capacity (DC); gene diversity (GD); match probability (MP); haplotype diversity (HD); sexual assault***

Introduction

DNA typing has been applied extensively in criminal casework. Autosomal short tandem repeat (STR) profiling is the most commonly used system [1], and commercial multiplex kits allow for the simultaneous analysis of more than 24 loci [2]. However, when the evidentiary item contains mixed DNA from two or more contributors, the resulting autosomal STR profile may exhibit complex mixtures for which the interpretation depends heavily on the analyst's experience. In addition, when the minor contributor is present at low template quantities, stochastic effects exacerbate the interpretation. This situation is particularly common in the evidence collected from victims of sexual assault (e.g., outerwear; underwear; vaginal, anal, or oral swabs;

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fingernail scrapings) [3]: the victim's cellular DNA often predominates, which may prevent the amplification of the male component or yield a male profile that is below the interpretation thresholds [4]. Y-chromosomal STR (Y-STR) typing targets male-specific markers and circumvents interference from the female DNA, thereby increasing the chance of recovering a male profile for comparison. Y-STRs are also informative in patrilineal kinship testing.

Because the Y chromosome is transmitted patrilineally as a nonrecombining haplotype, males from the same paternal line generally share identical Y-STR haplotypes, except when mutations occur. Consequently, Y-STRs cannot achieve the level of individualization provided by autosomal STRs; a match at Y-STRs does not by itself identify the source of the DNA within a paternal lineage. In addition, due to the low DNA quantity or quality, partial Y-STR profiles are common in casework, necessitating cautious interpretation. In certain low-quantity male Y-chromosome samples, only a partial Y-STR profile (for example, only a subset of Y-STR loci) may be detected. This can pose significant challenges for analysts during profile interpretation, resulting in uncertainty in the usability or reportability of the results. The interpretation is highly sensitive to the peak height of the detected alleles. A marginally low peak height increases the risk of false-negative conclusions due to allelic drop in/drop out caused by stochastic effects, even if the allele surpasses the AT. Conversely, very low peak heights increase the ambiguity, potentially leading to erroneous interpretations in which true alleles are mistakenly rejected, or vice versa. This variability in peak signal presents a persistent challenge to analysts. In such scenarios, the availability of a reference standard may provide analysts with considerable assistance and guidance.

We previously established and validated a 17-locus Y-STR system (AmpFLSTR™ Yfiler™ PCR Amplification Kit, Applied Biosystems, Foster City, CA, USA). Two more kits have been implemented in recent years: the PowerPlex® Y23 System and the Yfiler™ Plus PCR Amplification Kit, containing 23 and 27 loci, respectively. Here, we evaluate these two kits by computing gene diversity (GD) [5], the number of unique haplotypes (UH) [6], discriminatory capacity (DC) [7, 8], match probability (MP) [9], and haplotype diversity (HD) [10], according to laboratory quality-assurance requirements for new reagents and methods.

Materials and Methods

Sample sources

The Criminal Investigation Bureau collected the reference samples in accordance with the DNA Sampling Act in Taiwan. The known paternal relatives were excluded. The PowerPlex® Y23 System (Promega Corporation, Madison, WI, USA) and the Yfiler™ Plus PCR Amplification Kit (Thermo Fisher Scientific, Waltham, MA, USA) were used to type 418 and 574 nonduplicate reference samples, respectively, and 1,200 casework items. We do not report the individual locus-level genotypes here; all results are summarized statistically.

DNA extraction and quantification

We extracted the DNA from blood or saliva using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) and the VIOGENE Blood and Tissue Genomic DNA Extraction Miniprep System (Viogene Biotek Corp., Taipei, Taiwan), following the manufacturers' protocols. The extracts were stored at 4 °C. We quantified male human DNA using the Quantifiler™ Y Human Male DNA Quantification Kit (Thermo Fisher Scientific) using real-time polymerase chain reaction (PCR) on an ABI PRISM® 7500 Sequence Detection System (Thermo Fisher Scientific) with SDS v1.0 software. We used the quantification results to determine the input template for PCR.

PCR amplification and capillary electrophoresis

Between 0.25 and 5 ng of DNA template was amplified based on the kit's recommendations. For the PowerPlex® Y23 System, the cycling conditions were as follows: 96 °C for 1 min; 30 cycles of 94 °C for 10s, 61 °C for 1 min, 72 °C for 30s; final extension at 60 °C for 20 mins; and hold at 4 °C. For the Yfiler™ Plus kit: 95 °C for 1 min; 30 cycles of 94 °C for 4s, 61.5 °C for 1 min; final extension at 60 °C for 22 mins; and hold at 4 °C. The amplicons were separated on an ABI PRISM® 3130 Genetic Analyzer and genotyped with GeneMapper ID® (Thermo Fisher Scientific) using the corresponding allelic ladders. All calls were independently reviewed.

Statistical analyses

For each kit, the custom scripts computed the number of UH observed, DC (DC = UH/N), MP, and HD. The locus-specific GD was computed as $n(1 - \Sigma p_i^2)/(n - 1)$ [11], where n is the sample size and p_i is the frequency of haplotypes carrying the i-th allele. To evaluate the performance with partial profiles, the loci were ordered by (i) ascending GD and (ii) empirical detection rate in casework; the top n loci (n = 8~23 for PPY23; n = 8~27 for YFP27) were used to recompute UH, DC, MP, and HD.

Results and Discussion

Discriminatory capacity across kits

We analyzed PowerPlex® Y23 (23 loci) and Yfiler™ Plus (27 loci). Because the two kits share 21 loci, we also evaluated the overlapping panel (OLP21) and, for historical context, the earlier 17-locus Yfiler™ kit (YF17).

Table 1. Summary statistics for the Y-STR kits and overlapping panels.

	PPY23	YFP27	OLP21	YF17
No. of Loci	23	27	21	17
No. of Samples (N)	418	574	970	970
# Haplotypes (UH)	409	574	926	870
n =1	400	574	890	801
n = 2	9		31	54
n = 3			3	9
n = 4			1	3
n = 5			1	1
n = 6				0
n = 7				0
n = 8				1
n = 9				1
Discriminatory capacity	0.978	1	0.955	0.897

PPY23 = PowerPlex® Y23 System (23-locus Y-STR panel); YFP27 = Yfiler™ Plus PCR Amplification Kit (27-locus Y-STR panel); OLP21 = 21-locus panel shared by PowerPlex® Y23 and Yfiler™ Plus; YF17 = AmpFLSTR™ Yfiler™ PCR Amplification Kit (17-locus Y-STR panel).

We compared the two Y-STR typing kits and calculated their DC (DC = UH/N). The Yfiler™ kit (17 loci) yielded a DC of 0.897 (N = 970), the PowerPlex® Y23 System (23 loci) achieved a DC of 0.978 (N = 418), and the Yfiler™ Plus PCR Amplification Kit (27 loci) produced a DC approaching 1 (N = 574). For the overlapping 21-locus panel shared by PowerPlex® Y23 and Yfiler™ Plus—evaluated on specimens successfully typed at all 21 loci across both kits (N = 970)—the DC was 0.955. Taken together, these findings indicate that newer male-specific Y-chromosomal STR kits substantially increase DC and, consequently, enhance the probative value of Y-STR results in certain sexual assault casework.

Gene diversity by locus and population comparison

We also compared the GD estimates for the different Y-STR kits with the values reported in other studies [12]. We observed modest interpopulation differences at certain loci, but the magnitudes were generally small. In addition, the loci classified as rapid-mutation markers (mutation rate > 1%) [13, 14] exhibited a higher GD than

other loci did, consistent with their known mutational properties. We also calculated the locus-specific GD for the loci contained in each of the two kits; as summarized in Table 2, a comparison of the loci shared by both kits revealed no appreciable differences in GD across the corresponding loci.

Table 2. GD across loci for YFP27, PPY23, and OLP21.

Locus	Gene Diversity		
	YFP27	PPY23	OLP21
DYS576*	0.755	0.782	0.768
DYS389I	0.555	0.557	0.556
DYS635	0.729	0.766	0.746
DYS389II	0.736	0.742	0.738
DYS627*	0.837	N/A	N/A
DYS460	0.664	N/A	N/A
DYS458	0.801	0.810	0.804
DYS19	0.694	0.670	0.681
Y_GATA_H4	0.551	0.557	0.552
DYS448	0.720	0.728	0.724
DYS391	0.344	0.383	0.357
DYS456	0.640	0.705	0.670
DYS390	0.674	0.669	0.672
DYS438	0.455	0.398	0.431
DYS392	0.676	0.658	0.671
DYS518*	0.873	N/A	N/A
DYS570*	0.805	0.809	0.807
DYS437	0.512	0.509	0.512
DYS385 (a and b)	0.862	0.851	0.857
DYS449*	0.863	N/A	N/A
DYS393	0.577	0.587	0.583
DYS439	0.655	0.670	0.661
DYS481	0.778	0.801	0.789
DYF387S1 (a and b)*	0.843	N/A	N/A
DYS533	0.506	0.549	0.524
DYS549	N/A	0.602	N/A
DYS643	N/A	0.603	N/A

*Rapid-mutation locus.
N/A indicates that the locus was not present in the panel and was therefore excluded from the calculations.
PPY23 = PowerPlex® Y23 System (23-locus Y-STR panel); YFP27 = Yfiler™ Plus PCR Amplification Kit (27-locus Y-STR panel); OLP21 = 21-locus panel shared by PowerPlex® Y23 and Yfiler™ Plus.

Subset analyses ordered by GD and by empirical detection rate

Based on the locus-specific GD values listed in Table 2, the loci within each kit were ordered from lowest to highest GD. We then selected, respectively, the 8~23 loci with the lowest GD (for example, the 12 Y-STR loci exhibiting the lowest GD values are, in ascending order of GD, as follows: DYS391, DYS438, DYS437, DYS533, Y_GATA_H4, DYS389I, DYS393, DYS549, DYS643, DYS392, DYS390, and DYS439) for the PowerPlex® Y23 System (PPY23) and the 8~27 loci with the lowest GD (for example, the 12 Y-STR loci exhibiting the lowest GD values are, in ascending order of GD, as follows: DYS391, DYS438, DYS533, DYS437, Y_GATA_H4, DYS389I, DYS393, DYS456, DYS439, DYS460, DYS390 and DYS392) for the Yfiler™ Plus PCR Amplification Kit (YFP27), and recomputed the number of UH, DC (DC = UH/N), MP (MP = Σp_i^2), and HD. Figure 2 summarizes the results.

Using only the eight lowest-GD loci, PPY23 yielded 222 distinct Y-STR haplotypes among 418 samples (DC = 0.531, MP = 0.011, HD = 0.992), whereas YFP27 yielded 256 distinct haplotypes among 574 samples (DC = 0.446, MP = 0.014, HD = 0.987). When the subset was expanded to the 12 lowest-GD loci, PPY23 produced 321 UH among 418 samples (DC = 0.768, MP = 0.0048, HD = 0.998), and YFP27 produced 423 UH among 574 samples (DC = 0.737, MP = 0.0047, HD = 0.997). These benchmarks can serve as practical reference points for setting interpretation criteria in the evaluation of the Y-STR profile.

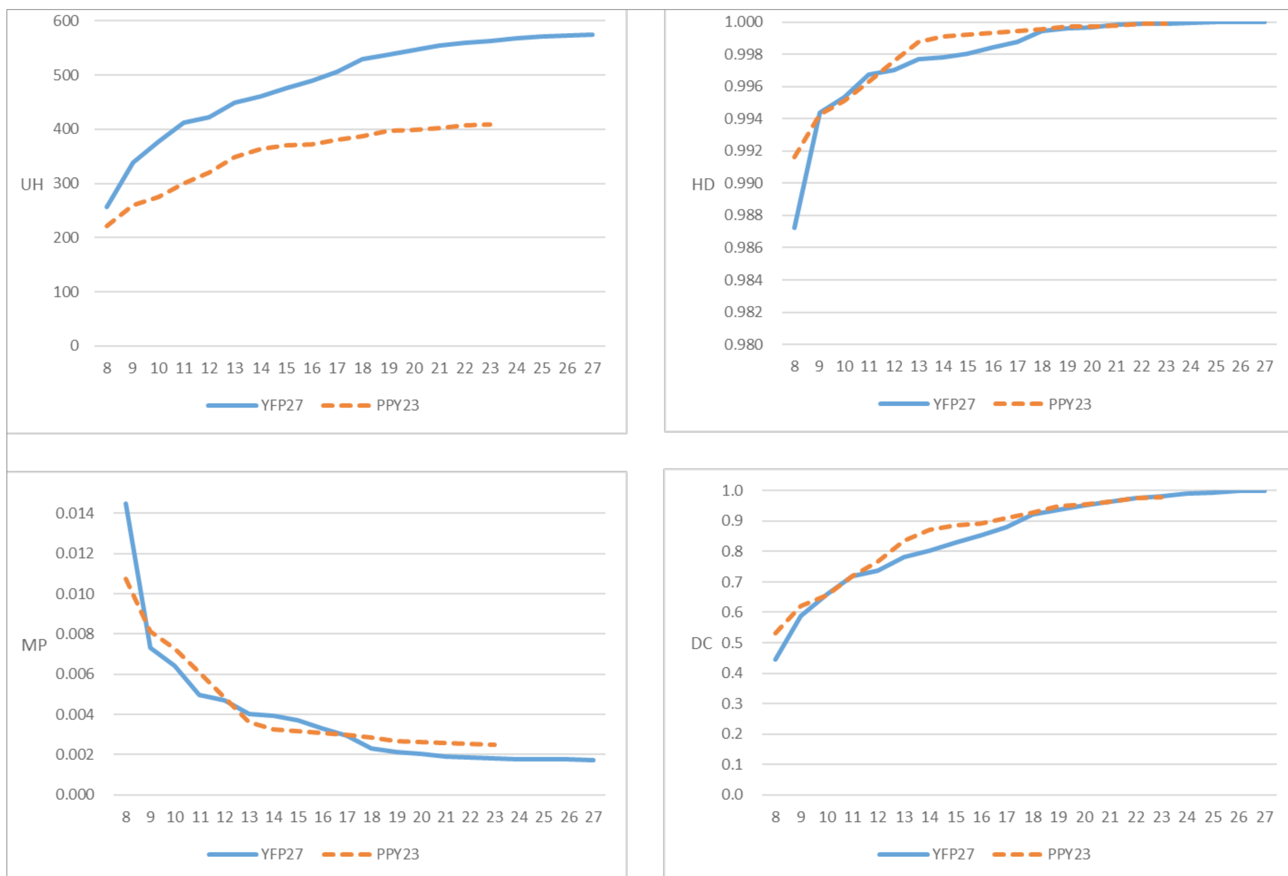

Fig 1. Comparative analyses of the PowerPlex® Y23 System and the Yfiler™ Plus PCR Amplification Kit using subsets of loci with the lowest locus-specific gene diversity (in ascending order)

x-axis: number (n) of lowest-GD loci selected (*n* = 8~27); *y*-axis: values of the computed statistics: UH = number of unique haplotypes; MP = match probability; HD = haplotype diversity; DC = discriminatory capacity.

Multiplex STR kits that use multiple sets of fluorescently labeled primers are subject to PCR competition, resulting in locus-to-locus differences in amplification efficiency. When DNA quality is compromised (e.g., fragmentation), loci with longer amplicons are more prone to low signal or dropout. In addition, low quantities of male DNA or the presence of inhibitors can induce stochastic effects that result in the nondetection at certain loci. Consequently, partial Y-STR profiles are frequently encountered in casework. To model the DC of such incomplete profiles, we used the PowerPlex® Y23 System and the Yfiler™ Plus PCR Amplification Kit to analyze evidentiary items that produced partial Y-STR results. For each kit, we computed per-locus detection rates from the casework (Table 3). In these calculations, loci that failed to meet validated acceptance thresholds were scored as "not detected"; we retained profiles for analysis if at least eight loci were detected. The numbers of samples tested (denominators) with partial Y-STR profiles were 170 and 1,030 for the PowerPlex® Y23 System and Yfiler™ Plus, respectively. Based on the detection-rate rankings presented in Table 3, we then selected the top 8~23 loci (PowerPlex® Y23) and the top 8~27 loci (Yfiler™ Plus) and then recomputed the number of UH, DC (DC = UH/N), MP (MP = Σp_i^2), and HD. Figure 1 presents the results.

Using the 12 loci with the highest detection rate, the PPY23 system yielded 394 distinct Y-STR haplotypes among 418 samples (DC = 0.943, MP = 0.0027, HD = 0.9997), whereas the YFP27 system yielded 524 distinct haplotypes among 574 samples (DC = 0.913, MP = 0.0023, HD = 0.9994). These benchmarks can serve as practical reference points when setting the interpretation criteria for Y-STR profile evaluation.

Table 3. Detection-rate ranking of samples with partial Y-STR profiles by the locus for YFP27 and PPY23.

Ranking of detection rate	YFP27 (n = 170)		PPY23 (n = 1,030)	
	Locus	Detection rate	Locus	Detection rate
1	DYS576	97%	DYS391	99%
2	DYS458	97%	DYS458	98%
3	DYS635	96%	DYS576	98%
4	DYS570	96%	DYS570	98%
5	DYS439	96%	DYS393	97%
6	DYS390	96%	DYS635	96%
7	DYS389I	95%	DYS481	95%
8	DYS19	95%	DYS549	95%
9	DYS438	95%	DYS390	95%
10	DYS460	95%	DYS438	93%
11	DYS456	95%	DYS448	93%
12	DYS437	95%	DYS533	93%
13	DYS448	94%	DYS389I	93%
14	Y GATA H4	94%	Y GATA H4	92%
15	DYS393	94%	DYS456	92%
16	DYS391	92%	DYS439	91%
17	DYS392	91%	DYS392	90%
18	DYS481	91%	DYS389II	89%
19	DYS627	88%	DYS437	88%
20	DYS449	88%	DYS19	87%
21	DYS533	88%	DYS385a	87%
22	DYS518	87%	DYS385b	87%
23	DYF387S1a	84%	DYS643	82%
24	DYF387S1b	83%	N/A	N/A
25	DYS389II	82%	N/A	N/A
26	DYS385a	79%	N/A	N/A
27	DYS385b	78%	N/A	N/A

N/A indicates the locus was not present in the panel and was therefore excluded from the calculations.

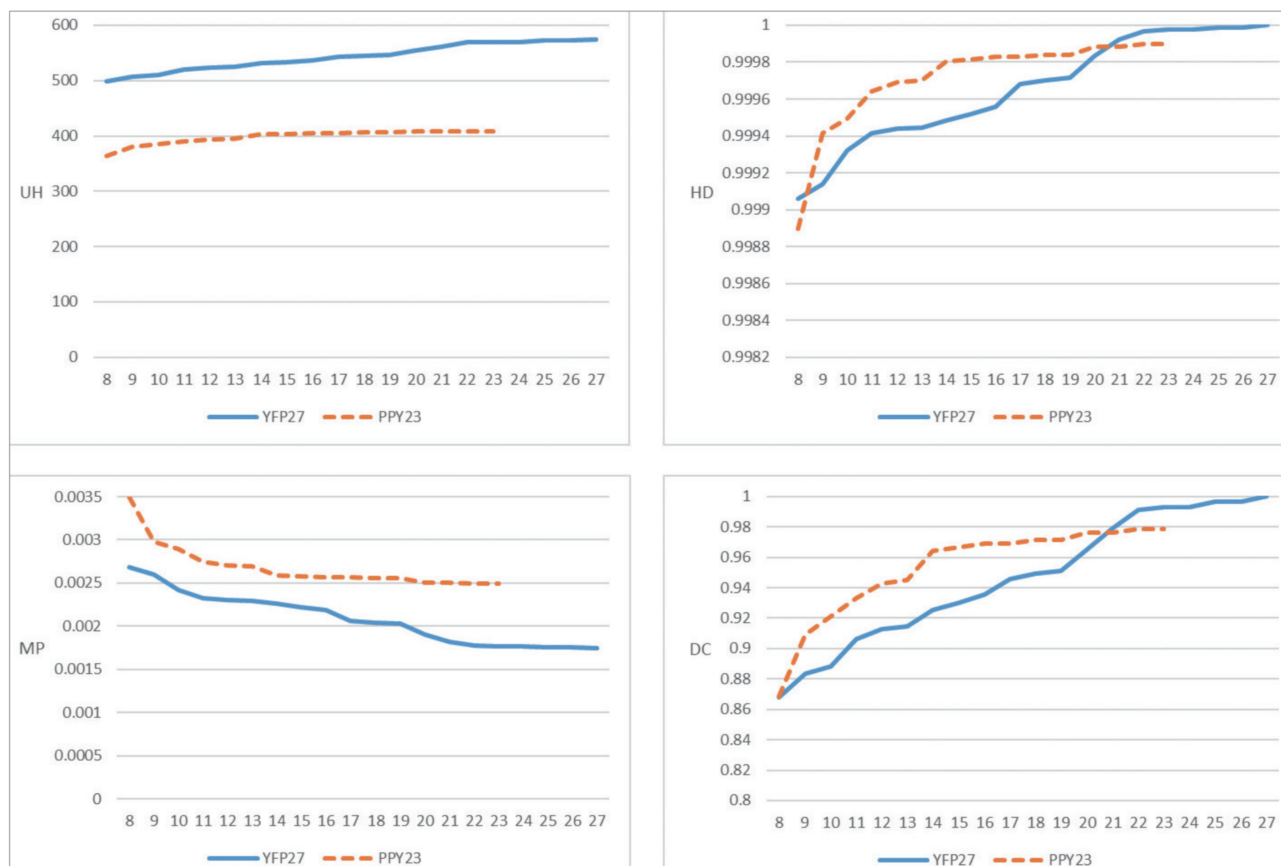

Fig 2. Comparative analyses of the PowerPlex® Y23 System and the Yfiler™ Plus PCR Amplification Kit using loci ranked by the locus-specific detection rate

x-axis: number (n) of highest-detection-rate loci selected (n = 8~27); *y*-axis: values of the computed statistics: UH = number of unique haplotypes; MP = match probability; HD = haplotype diversity; DC = discriminatory capacity.

Conclusion

Both the PowerPlex® Y23 System and the Yfiler™ Plus PCR Amplification Kit achieved high DC. Because the Y chromosome is inherited as a patrilineal haplotype, complete individualization is not feasible. Furthermore, while interpreting the Y-STR results, the possibility must be considered that the profile may originate not only from the individual himself or his paternal relatives but also from an unrelated random male. However, including the rapid-mutation loci in both kits increased their DC, a finding confirmed by our analyses. To assist in interpreting the partial profiles, we examined the subsets of loci ordered by (i) the lower GD and (ii) the higher empirical detection rates. For example, using the first 12 loci, PPY23 yielded 321 UH among 418 samples (DC = 0.768, MP = 0.0048, HD = 0.998), and YFP27 yielded 423 among 574 samples (DC = 0.737, MP = 0.0047, HD = 0.997). With reference sample sizes of 418 and 574, these estimates are statistically meaningful according to different views of the loci. These results can be used by forensic laboratories as a reference for Y-STR profile interpretation. However, to establish suitable reference standards, the forensic laboratories must conduct internal analysis and validation based on their specific reagents, instrumentation, and methodologies. Regardless of the final number of loci used by the laboratory for the interpretive analysis or the actual number of loci reported in the final documentation, it is still advised that the haplotype random MP be calculated by referencing the SWGDAM guideline document (SWGDAM Interpretation Guidelines for Y-Chromosome STR Testing) or other established/published methods, providing a valuable resource for both the submitting

agencies and the relevant judicial bodies. Future work with larger datasets may refine these population parameters and further improve the interpretation guidelines for evidentiary Y-STR profiling.

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