

21 STR LOCI for INCREASING PATERNITY INDEX AND PATERNITY PROBABILITY

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Abstract

DNA testing has become a formal and reliable method for paternity testing. There are many commercial kits available for human identification which is commonly used in routine analysis. However, there is no commercial kit that is produced specifically for paternity testing purpose. Currently, AmpFLSTR® Verifiler™ Direct PCR Amplification kit was developed for paternity using only. The kit can amplify 9 autosomal STR loci, D10S1248, D1S1656, D2S1338, D22S1045, D19S433, TH01, D2S441, D6S1043, D12S391, and the sex determining marker, Amelogenin. From our results of low combined paternity index (PI) cases when using Verifiler™ Direct kit, the combined PI and W values showed higher combined PI and W values than those from using Identifiler® kit. For mutation cases if the mutated locus in Verifiler™ Direct kit was the same locus in Identifiler® kits, the combined PI and W from Verifiler™ Direct kit was less than those from Identifiler® kit. On the other hand if the mutated locus occurred only in Identifiler® kit, the DNA profiles generated from Verifiler™ Direct kit will have higher combined PI and W than those from Identifiler® kit. Finally, combination of both Verifiler™ Direct and Identifiler® kits showed the total combined PI and W with higher values than using one kit. The total combined PI ranged from 10^3 - 10^7 , and W values ranged from 99.9 – 99.99999%. For conclusion, Verifiler™ Direct kit was used in conjunction with the Identifiler® kit to provide a total of 21 unique STR loci for increasing PI and W values. Therefore, the Verifiler™ Direct kit is a useful tool for increasing statistical values of the interpretation of paternity testing.

Keywords : Paternity testing, Short Tandem Repeats (STR), Verifiler™ Direct kit, Combined paternity index (PI), Probability of paternity (W)

เครื่องหมายพันธุกรรม STR 21 ตำแหน่งในการเพิ่มค่าดัชนีและโอกาสความเป็นบิดา

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บทคัดย่อ

การตรวจพิสูจน์ดีเอ็นเอเป็นวิธีการที่มีความน่าเชื่อถือสำหรับการตรวจพิสูจน์ความเป็นบิดา แต่สำหรับห้องปฏิบัติการตรวจวิเคราะห์ดีเอ็นเอนั้น ยังไม่มีชุดน้ำยาเพิ่มปริมาณดีเอ็นเอที่ผลิตมาใช้โดยเฉพาะสำหรับการตรวจพิสูจน์ความเป็นบิดา ปัจจุบันมีชุดน้ำยาสำเร็จรูปคือ AmpFLSTR® Verifiler™ Direct PCR Amplification Kit ถูกผลิตขึ้นมา โดยมีวัตถุประสงค์เพื่อใช้ในการตรวจพิสูจน์ความเป็นบิดาโดยเฉพาะ ซึ่งชุดน้ำยานี้สามารถเพิ่มปริมาณดีเอ็นเอบริเวณ STR ได้จำนวน 9 ตำแหน่งบนออโตโครโมโซมและอีก 1 ตำแหน่งบนโครโมโซมเพศ ได้แก่ D10S1248, D1S1656, D2S1338, D22S1045, D19S433, TH01, D2S441, D6S1043, D12S391 และ Amelogenin จากรายงานนี้พบว่า เมื่อใช้ Verifiler™ Direct kit ในตัวอย่างที่มีค่าดัชนีความเป็นบิดาต่ำพบว่าทั้งค่าดัชนีและค่าโอกาสความเป็นบิดาจะมีค่าเพิ่มขึ้นสูงกว่าค่าดังกล่าวจากการใช้ Identifiler® kit เพียงชนิดเดียว สำหรับตัวอย่างที่มีการกลายและตัวอย่างที่มีทั้งการกลายและค่าดัชนีความเป็นบิดาต่ำพบว่า หากตำแหน่งที่เกิดการกลายของ Verifiler™ Direct kit เป็นตำแหน่งเดียวกับ Identifiler® Kit การกลายนั้นจะส่งผลกระทบต่อค่าดัชนีและค่าโอกาสความเป็นบิดา ในทางตรงกันข้าม ถ้าตำแหน่งที่เกิดการกลายเป็นตำแหน่งที่มีเพียงใน Identifiler® kit รูปแบบดีเอ็นเอที่ได้จากการการใช้ Verifiler™ Direct kit จะให้ค่าดังกล่าวที่สูงกว่าการใช้ Identifiler® kit แต่เมื่อวิเคราะห์ดีเอ็นเอโดยใช้น้ำยาสำเร็จรูปทั้ง 2 ชนิดพบว่า ค่าดัชนีความเป็นบิดาของทั้ง 10 กรณีศึกษาจะมีค่าเพิ่มมากขึ้น อยู่ในช่วง 103 - 107 และค่าโอกาสความเป็นบิดาจะมีค่าสูงขึ้นอย่างสอดคล้องกันระหว่างร้อยละ 99.9 - 99.99999 ซึ่งค่าดังกล่าวจะสูงกว่าค่าที่ได้รับจากการใช้ Identifiler® Kit เพียงชนิดเดียว สรุปได้ว่า การรวมกันของค่าดัชนีความเป็นบิดาจากการใช้ Verifiler™ Direct kit และ Identifiler® Kit ทั้งหมด 21 ตำแหน่งของเครื่องหมายพันธุกรรม STR สามารถเพิ่มค่าดัชนีและค่าโอกาสความเป็นบิดาให้สูงขึ้นได้ ดังนั้น Verifiler™ Direct kit จึงเป็นอีกทางเลือกหนึ่งที่สามารถนำมาใช้ให้เกิดประโยชน์ในการเพิ่มค่าทางสถิติที่เกี่ยวข้องกับการตรวจพิสูจน์ความเป็นบิดา และส่งผลให้การแปลผลนั้นมีความน่าเชื่อถือมากยิ่งขึ้นในเชิงสถิติ

คำสำคัญ : การพิสูจน์ความเป็นบิดา ดีเอ็นเอชุดซ้ำ(เอสทีอาร์) ชุดน้ำยาสำเร็จรูปเวอร์ริไฟเลอร์ไดเร็ก ดัชนีความเป็นบิดา โอกาสความเป็นบิดา

1. Introduction

A paternity testing is to determine that a man is the biological father of another person. In the past, some methods were used for paternity testing such as ABO blood group, human leukocyte antigen (HLA) or analysis of various proteins and enzymes. Nowadays, DNA testing has become a formal and reliable method for paternity testing [3]. Therefore, there are many commercial kits available for human identification which is commonly used in routine analysis. Nevertheless, there is no commercial kit that was produced specifically for paternity testing purpose. Currently, AmpFLSTR® Verifiler™ Direct PCR Amplification kit is a new commercial kit for paternity test using only. The kit provides simultaneous amplification of 9 autosomal Short Tandem Repeats (STR) loci : D10S1248, D1S1656, D2S1338, D22S1045, D19S433, TH01, D2S441, D6S1043, D12S391, and the sex determining marker as Amelogenin. The Verifiler™ Direct kit was optimized for application with Identifiler® kits in order to increase the discriminatory power when a paternity test is difficult, such as, low paternity index (PI) value and cases involving mutational events [1]. Therefore, the purpose of this study was to use Verifiler™ Direct kit in conjunction with Identifiler® kit to increase the discrimination power of paternity cases in routine investigation.

2. Materials and methods

2.1 Sample collection

DNA of 10 families was left-over specimen from Human Genetics Unit, Ramathibodi Hospital. These families were divided into 3 groups: (1) low combined paternity index (PI) cases, 4 duo families, (2) mutation cases, 1 trio and 2 duo families, and (3) low CPI and mutation cases, 3 duo families.

2.2 PCR amplification

Twenty one samples were amplified using AmpFLSTR® Verifiler™ Direct PCR Amplification Kit. 10 µL of Verifiler™ Direct Master Mix, 5 µL of Verifiler™ Direct Primer Set and 10 µL of DNA samples were mixed in each reaction well of 0.2 mL PCR 8-Strip tubes. The PCR reaction amplified using GeneAmp® PCR System 9700 with gold-plated silver block. The amplification

immediately place on ice for 3 minutes. Then the PCR products were subjected to a capillary electrophoresis (Applied Biosystems® 3500/3500xL Genetic Analyzers). The Data Collection Software stores information for each sample in an .fsa file by using GeneMapper® ID-X Software v1.3 software to analyze and interpret the results [1].

2.4 Statistical analysis

DNA profiles of 10 families were calculated for a combined paternity index (PI) and a probability of paternity (W) value [2]. Then, the CPI values of the data generated from Verifiler™ Direct kit were combined with those from Identifiler® kit. Finally, the total combined PI of Verifiler™ Direct kit and Identifiler® kit was subsequently used to calculate the paternity probability (W) of each family [2].

3. Result and Discussion

A total of 10 families were selected to be the case study. The DNA profiles using Verifiler™ Direct kit were calculated for the combined PI and W values (Table 1). For low combined paternity index (low combined PI) cases (family No. 21, 194, 315 and 459), the combined PI and W values from 9 STR loci generated by Verifiler™ Direct kit were higher than those from 15 STR loci using Identifiler® kit.

For mutation cases, family No. 50 mutated at D18S51 and D21S11 that were not included in the Verifiler™ Direct kit. When using Verifiler™ Direct kit, the combined PI and W values increased from 2,618.013 to 21,296.571 ($W = 99.925$ to 99.995%). Nevertheless, family No. 250 and 100 showed mutation at D2S1338 and D19S433, respectively. Both D2S1338 and D19S433 are included in Verifiler™ Direct kit. When using Verifiler™ Direct kit, combined PI and W values decreased from 11,424.423 to 0.447 ($W = 99.991$ to 30.888%) in family No. 100, and decreased from 7,222.923 to 167.233 ($W = 99.986$ to 99.406%) in family No. 250.

Table 1 Combined paternity index (PI) and paternity probability (W) values from DNA profiles using Verifiler™ Direct kit in conjunction with Identifiler® kit of low paternity index and mutation cases

Group	Family No.	Mutation	Identifiler (15 loci)		Verifiler (9 loci)		Identifiler+Verifiler (21 loci)	
			Combined PI	W (%)	Combined PI	W (%)	Combined PI	W (%)
Low combined PI	21 (duos)	-	123.449	99.196	206.616	99.518	2,779.292	99.96
	194 (duos)	-	440.277	99.773	231,178.284	99.9996	25,834,706.800	99.999996
	315 (duos)	-	221.992	99.552	29,388.472	99.997	3,384,845.917	99.99997
	459 (duos)	-	33.417	97.094	1,739.254	99.943	13,284.285	99.992
Mutation	50 (trios)	D18S51 D21S11	2,618.013	99.93	21,296.571	99.995	6,934,276.323	99.99999
	100 (duos)	D19S433	11,424.423	99.991	0.447	30.888	340,385.652	99.9997
	250 (duos)	D2S1338	7,222.923	99.99	167.233	99.406	4,983,857.183	99.99998
Low combined PI and mutation	193 (duos)	D2S1338	5.289	84.100	1.565	61.016	1,013.373	99.90
	255 (duos)	D2S1338	39.704	97.543	3.541	77.978	2,783.024	99.96
	569 (duos)	D3S1358	109.800	99.097	2,465.955	99.959	45,956.500	99.998

The results of low combined PI and mutation cases were consistent with the results of mutation cases. Both family No. 193 and 255 mutated at D2S1338 that was included in Verifiler™ Direct kit. When using Verifiler™ Direct Kit, the combined PI and W values decreased from 5.289 to 1.565 ($W = 84.100$ to 61.016%) in family No. 193 and decreased from 39.704 to 3.541 ($W = 97.543$ to 77.978%) in family No 255. However, family No. 569 had mutation at D3S1358 that was not included in Verifiler™ Direct kit. When using Verifiler™ Direct kit, the combined PI and W values increased from 109.800 to 2,465.955 ($W = 99.097$ to 99.959%)

From the results of statistically difficult paternity cases, the data showed that both low combined PI values and mutational events had an effect on probability of paternity (W). If the mutated locus in Verifiler™ Direct kit was the same locus in Identifiler® kit, the combined PI and W from Verifiler™ Direct kit was less than those from Identifiler® kit. On the other hand if the mutated locus occurred only in Identifiler® kit, the DNA profiles generated from Verifiler™ Direct kit will have higher combined PI and W than those from Identifiler® kit. Although, Verifiler™ Direct kit was used in mutation cases, the combined PI and W values did not increase but those values were also less than the values from using Identifiler® Kit.

When two kits were used together, Verifiler™ Direct and the Identifiler® kits allowed the amplification of 21 high discriminatory STRs plus amelogenin marker. The combined PI and W values of 15 STR loci in Identifiler® kit were combined with those of 6 STR loci in Verifiler™ Direct kit. The PI values of D2S1338, D19S433 and TH01 loci in Verifiler™ Direct kit was not calculated with the combined PI of 15 STR loci in Identifiler® kit. The combined PI and W values of all families that were selected to be the case study increased after conjugation with Verifiler™ Direct kit. The combined PI of 10 cases ranged from 10^3 - 10^7 , and the paternity probability (W) ranged from 99.9 – 99.99999%.

When statistical values generated by Verifiler™ Direct kit was combined with those from Identifiler® kit, the minimum combined PI and W values of each group showed that the combined PI and W values of family No. 459 increased from 33.417 to 13,284.285 ($W = 97.094$ to 99.992%) for low CPI cases. For mutation cases, the combined PI and W values of family No. 50 increased from 2,618.013 to 6,934,276.323

($W = 99.93$ to 99.99999%). For low combined PI and mutation cases, the combined PI and W values of family No. 193 increased from 5.289 to 1,013.373 ($W = 84.100$ to 99.90%) (Figure 1).

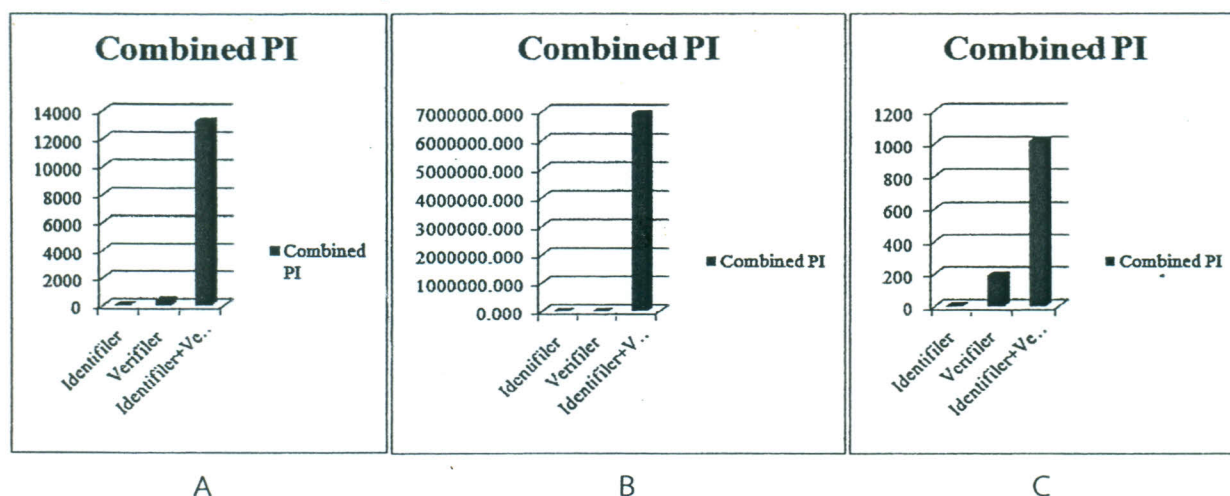


Figure 1 Combined PI from using Identifiler[®] kit, Verifiler[™] Direct Kit and the combination of both kits. A : family No. 459(duos), B : family No. 50(trios) and C : family No. 193 (duos)

4. Conclusion

DNA samples of 10 case studies were amplified using Verifiler[™] Direct kit. After that the combined PI values of the Verifiler[™] Direct kit was combined with the combined PI values of the Identifiler[®] kit, the results showed that the total combined PI and W values increased in all cases. Our data suggested that combination of Verifiler[™] Direct kit and Identifiler[®] kit to provide a total of 21 unique STR loci can increase the combined PI and W values. Moreover, 4 loci overlapping including D2S1338, D19S433, TH01 and Amelogenin can be used as internal controls. For summary Verifiler[™] Direct kit is a useful tool for increasing the confidence for the interpretation of paternity testing.

5. References

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