Validation Study of 6 Dye-Based SF25™ PCR Amplification Kit

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Abstract

Ever since the inception of STR-based forensic DNA analysis for human identification and DNA profiling, the approach developed continuously. Most of these developments are pertaining to achieve the best polymorphic loci set for analysis, high inhibitor tolerance capability, increase in sensitivity, reduction in turnaround time, and cost-effectiveness. On the similar line, the SF25TM PCR amplification kit, a 6 fluorescent dye-chemistry based autosomal STR kit was developed, which analyses 25 STR loci viz., FAM fluorescent dye-labelled D3S1358, D13S317, D7S820, D16S539, D1S1656, and Penta E; HEX fluorescent dye-labelled TPOX, TH01, D2S1338, CSF1PO, and Penta D; SUM fluorescent dye-labeled D19S433, vWA, D21S11, D18S51, and D6S1043; LYN fluorescent dye-labelled Amelogenin, D8S1179, D5S818, D12S391, and FGA; PUR fluorescent dye-labelled D22S1045, Y indel; D2S441 and D10S1248. The developmental validation of the SF25 PCR Amplification reagents were carried out in this study to analyze its specificity, sensitivity, and concordance. This multiplex kit showed higher sensitivity with 125pg of DNA template generating a complete profile (with 100% allele call). Out of 83 samples, 10 samples showed aberrant triallelic pattern/aberrant heterozygous pattern at D16S539 locus. The possible discrepant homozygous condition at locus D1S1656 might be due to the invasion of alleles at D1S1656 locus present adjacent to D16S539 locus. Such invasion resulted due to the shortening of the allelic bin at the D1S1656 locus. The allelic ladder of the SF25TM PCR amplification kit reveals the allelic range of D1S1656 from 11 to 19.3 which can be interpreted according to the casework as deemed by the end-users for their samples.

Introduction:

Short Tandem Repeats (STRs) have become the most predominating polymorphic markers for paternity testing, individual identification, and database management for criminal investigations on account of their genomic abundance, co-dominance nature, and existence in low-copy regions¹. The ever-growing requirements for the development of competent, efficient, highly discriminative DNA typing techniques and reagent systems, with minimal adventitious matches, are conforming with the Combined DNA Index System (CODIS) and the European Standard Set (ESS). The requirement of innovation in the STR-based kits was to bring in expansion in the number of core loci, that would help obtain additional information on the polymorphic loci². The move to expand the list of Combined DNA Index System (CODIS) markers from CODIS-13 to CODIS-20 by the Federal Bureau of Investigation (FBI) and by European Standard Set (ESS) from ESS from 7 markers to ESS 12 markers manifest the importance of incorporating additional loci, to the core loci, for an enhanced discriminatory power³⁻⁵ and to ensure more accurate human identification.

The SF25TM PCR amplification kit (Sorenson Genomics, USA) is a 6-dye chemistry-based multiplex STR system that allows amplification of 25 autosomal STR loci which encompasses recommended CODIS 20 and ESS 12 loci (TH01, TPOX, CSF1PO, FGA, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, D22S1045), other highly discriminating routinely tested loci Penta D, Penta E, D6S1043, along with the sex-determining marker amelogenin and Y-specific insertion-deletion locus Y-indel⁶ (Table 1).

Table 1
SF25™ PCR amplification kit locus specific information

STR locus	Repeat numbers in allelic ladder	Chromosomal position	Dye
D3S1358	11,12,13,14,15,16,17,18,19,20	3p21.31	FAM
D13S317	6,7,8,9,10,11,12,13,14,15	13q31.2	
D7S820	6,7,8,9,10,11,12,13,14,15	7q21.11	
D16S539	6,7,8,9,10,11,12,13,14,15	16q24.1	
D1S1656	11,12,13,14,15,16,17,17.3,18.3,19.3	1q42	
Penta E	5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25	15q26.2	HEX
TPOX	6,7,8,9,10,11,12,13,14	2p25.3	
TH01	4,5,6,7,8,9,9.3,10,11,13.3	11p5.5	
D2S1338	13,14,15,16,17,18,19,20,21,22,23,24,25,26,27	2q35	
CSF1PO	7,8,9,10,11,12,13,14,15	5q33.1	
Penta D	2.2,5,6,7,8,9,10,11,12,13,14,15,16,17	21q22.3	SUM
D19S433	9,10,11,12,12.2,13,13.2,14,14.2,15,15.2,16,16.2,17,17.2	19q12	
vWA	12,13,14,15,16,17,18,19,20,21,22	12p13.31	
D21S11	25,26,27,28,28.2,29,29.2,30,30.2,31,31.2,32,32.2,33,33.2,34,34.2,35,35.2,36,37	21q21.1	
D18S51	9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28	18q21.33	
D6S1043	9,10,11,12,13,14,15,16,17,18,19,20,21,21.3	6q15	LYN
Amelogenin	X, Y	p22.1-22.3/p11.2	
D8S1179	8,9,10,11,12,13,14,15,16,17,18	8q24.1	
D5S818	7,8,9,10,11,12,13,14,15	5q23.2	
D12S391	15,16,17,18,19,20,21,22,23,24,25,26,27,28	12p12	
FGA	17,18,19,20,21,22,23,24,25,26,27,28,29,42.2,43.2,44.2,45.2,46.2,47.2,48.2,49.2,50.2	4q28	PUR
D22S1045	11,14,15,16,17,18,19	22q12.3	
Yindel	1,2	Yq11.221	
D2S441	7,9,10,11,12,13,14,15	2p14	
D10S1248	8,11,12,13,14,15,16,17,18	10q26.3	

Laboratory evaluation and validation of SF25™ PCR amplification kit (Sorenson Genomics, LCC, salt lake city, USA) was performed following the guidelines recommended by Federal Bureau of Investigation (FBI) Quality Assurance Standards (QAS) 8.3.1⁷, ISO/IEC 17025: 2017 Standard 7.2.2⁸ and the Scientific Working Group on DNA Analysis Methods (SWGDM)⁹ in terms of precision, sensitivity, dynamic range, detection limits, stability, reproducibility, PCR issue related studies, concordance studies, male-female DNA mixture analysis, case-type samples testing and inhibitor tolerance study. The validation substantiated the robustness, stability, sensitivity, and reliability of the SF25™ PCR amplification kit for forensic applications.

Materials And Methods:

Sample preparation:

Genomic DNA was extracted using the organic extraction method and EZ1 DNA Investigator Kit- (QIAGEN)¹⁰, DNA extraction was carried out as per the manufacturer's recommended protocol. To evaluate the sensitivity and dynamic range of performance of the kit being studied, over a range of varied DNA concentrations, serial dilution of the control DNA 2800M (Promega, USA) were made for the analysis, triplicates of each set of serially diluted DNA were analyzed as 1000pg, 500pg, 250pg, 125pg, 62.5pg, 31.25pg, 15.6pg and non-template control (NTC). For the mixture study, AmpF/STR® control male DNA 007 (Thermo Fisher Scientific, USA) and AmpF/STR® control female DNA 9947A were mixed according to their concentration in a prescribed ratio. For the concordance study, forensic paternity casework (blood) samples were used. Non-probative samples were quantified on 7500 Real-Time PCR System (Thermo Fisher Scientific, USA)¹¹ using PowerQuant® system (Promega, USA) before PCR amplification.

Marker Characterization:

25 STR loci namely D3S1358, D13S317, D7S820, D16S539, D1S1656, Penta E, TPOX, TH01, D2S1338, CSF1PO, Penta D, D19S433, vWA, D21S11, D18S51, D6S1043, D8S1179, D5S818, D12S391, FGA, D22S1045, D2S441, D10S1248, amelogenin and Y-indel included in SF25™ PCR amplification kit comprise of all the recommended core loci in expanded CODIS (CODIS 20) and European Standard Set (ESS). Characteristics of each locus were listed in Table 1.

Multiplex Amplification and thermal cycling parameters

PCR reaction system for validation was performed with a full reaction volume of 25 µL,a reduced reaction volume of 12.5 µL, and a reduced reaction volume of 6 µL. The PCR reaction volumes of the respective components are shown in Table 2. Amplification was performed in Veriti™ 96-Well Thermal Cycler (Thermo Fisher Scientific, CA, USA)¹² with the following thermal cycling parameters: initial incubation at 95°C for 5 min; 29 annealing cycles of 94°C for 15 sec, 59°C for 40 sec, and 63°C for 70 sec; final extension at 60°C for 5 min and final hold at 4°C.

Table 2: Details of PCR reaction volumes			
	25 µLPCR Reaction volume	12.5 µL PCR reaction volume	6 µL PCR reaction volume
Super Mix (in µL)	10	5	2.5
SF25 Primers (in µL)	5	2.5	1.25
DNA template (containing DNA 0.2 ng/ µL)	5	2.5	1.25
Amplification grade water to achieve final reaction volume (in µL)	5	2.5	1
Final reaction volume (in µL)	25	12.5	6

Effect of PCR cycle no., different thermal cyclers, M:F ratio and other PCR related validation studies were carried out with 25 µL /12.5 µL /6 µL reaction volume.

Genotyping and Data Analysis

Capillary electrophoresisof amplified DNA products was carried out on Genetic Analyzer 3500XL (Thermo Fisher Scientific, CA, USA)¹³. J6 dye matrix set was used, which includes five different fluorescent dyes (FAM for blue, HEX for green, SUM for yellow, LYN for red, PUR for Purple) for labeling STR loci (Fig. 1), and orange fluorescent dye SIZ for labeling internal DNA size standard. The reagent cocktail used for CE is composed of 12µL of Hi-Di™ formamide and 0.5 µL size standard - Marker SIZ-500 for each reaction. Samples for analysis were prepared by the addition of 1 µL of PCR product to 12.5 µL of formamide and size standard reagent cocktail on a 96 well PCR plate. The sample plate was centrifuged at 3000rpm for 1 min and subjected to denaturation at 95°C for 3 minutes and ice-chilled immediately. Samples were injected at 1.2KV for 10 seconds. Data analysis and genotyping were performed using GeneMapper ID-X Software v1.5 (Thermo Fisher Scientific, CA, USA) with a threshold for allele peak calls at 175 RFU. All the suggested laboratory standards were followed during the present study.

Results And Discussion:

Sensitivity and variant PCR reaction volume

Complete DNA profiles (100% alleles called) were obtained with 25µL, 12µL, and 6µL PCR reaction volumes when the input amount of DNA template was 125pg (Fig. 2), which shows high sensitivity of the studied multiplex PCR kit. When DNA input was 62.2pg, 85.71% of alleles (42/49) were called, at a DNA input of 31.25pg and 15.62 pg, the alleles called were observed at 55.10% (27/49) and 42.86% (21/49) respectively (Fig. 3-5). On amplifying 1 ng of DNA, the average peak height ratio was 0.986, which increased to 0.995 on increasing the DNA input to 2ng.A gradual decrease in peak height was observed from 500 pg to 15.62pg input DNA, resulting in peak imbalance (Fig. 6). Here it is clarified that the alleles drop out at particular loci were not included in the peak height ratio analysis.

Effect of different PCR cycle numbers:

Recommended PCR cycle number can be increased to enhance the amplification signal of low copy DNA¹⁴ or decreased to reduce the turnaround time when abundant DNA sample¹⁴ is available. In direct amplification the amount of sample varies from sample types, therefore representative samples should be evaluated for optimal PCR cycle number. In the present study, the SF25™ PCR amplification kit was studied for different PCR cycle numbers 23 to 32, and peak heights were compared to recommended 29 PCR cycle protocol. As expected, peak height increased as the increased PCR cycle number (Fig.7). In general, more than 30 PCR cycle numbers produced artifacts, imbalanced peak heights, dropouts, drop-ins were observed for low copy DNA samples. In this study, imbalanced peak heights were observed at 32 PCR cycle numbers (Fig. 8).

Effect of different thermal cycler:

It is observed that the different laboratories used different make and model of a thermal cycler, therefore to evaluate the robustness of studied multiplex kit with various model/brand of thermal cycler namely, Veriti™ 96 well PCR system (Thermo Fisher Scientific, CA, USA),GeneAmp PCR System 9700 with Gold-plated 96-Well Block (Thermo Fisher Scientific, CA, USA), ProFlex™ PCR system (Thermo Fisher Scientific, CA, USA), Prima-DUO™ Thermal Cycler (HiMedia Laboratories India), Eppendorf Mastercycler® nexus (Eppendorf AG, Hamburg, Germany) were used.To overcome stochastic effects, the DNA profiles obtained from less than 500pg were considered for interlocus balance study. No significant variation (p<0.05) was observed in the interlocus balance of DNA profiles obtained using the aforementioned thermal cycler at variant DNA quantity (Fig. 9). To examine global balance (mean peak heights of same fluorescent-labeled dye were analyzed) using various thermal cycler, expectedly no significant variations observed (Fig. 10).

Male-Female DNA mixtures study:

In forensic casework, evidence that contains biological materials originating from more than one source is generally encountered. Studies related to mixtures can assist in mixture interpretation, the number of contributors, minor and major contributors, and their ratios. To correctly interpret results from DNA mixtures (male and female), it is important to realize the limitations of minor contributing alleles. Samples were prepared by mixing AmpF/STR® control male DNA 007 (Thermo Fisher Scientific, USA) and AmpF/STR® control female DNA 9947A for a total of 1 ng of template DNA in the ratios (male DNA: female DNA) of 1:0, 19:1, 9:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:9, 1:19, and 0:1. Each mixture set was tested in a triplicates. On average, 100% of alleles at 9:1 ratio as shown in Table 3 and the corresponding Figure 11. When the difference in mixture ratio was more, there was a decrease in the percentage of minor alleles that could be identified and as a result, partial profiles were yielded. This finding support high sensitivity and great importance in forensic application.

Table 3: Male: Female DNA mixture Ratio			
Male: Female Ratio	No. of Alleles called	Total Number of Alleles	Percentage of Profile
19:1	56	78	71.79
9:1	78	78	100
5:1	78	78	100
2:1	78	78	100
1: 2	78	78	100
1:5	78	78	100
1:9	76	78	97.44
1:19	60	78	76.92

Forensic sample analysis:

Challenging casework samples are generally encountered at the crime scene. These samples potentially possess inhibitory factors as well as degradation due to environmental exposure, resulting in low DNA yield. In order to test for the efficiency of the SF25™ PCR amplification kit we carried out this analysis with DNA samples obtained from the crime scene such as blood-stained exhibits, semen, and vaginal fluid stains, multiple tissues (muscles, bone, tooth) to evaluate the ability to obtain a DNA profile. The SF25™ PCR amplification kit profiling results obtained by analyzing 10 forensic casework samples showed full concordance with previous results generated by PowerPlex Fusion 6C¹⁵ (data not shown). All the challenging samples rendered full profiles with both SF25™ PCR amplification kit and PowerPlex® Fusion 6C system (Fig.12).

Concordance Studies:

A concordance study of the SF25™ PCR amplification kit was carried out to determine the accuracy of allelic designations on Control DNA 2800M and 83 other samples with PowerPlex® Fusion 6C system¹⁶. Out of 83 samples, profiles of 10 samples revealed the presence of false triallelic or false heterozygous pattern at D16S539 locus and simultaneously producing false homozygous condition at locus D1S1656 due to invasion of alleles at D1S1656 locus to adjacent D16S539 locus (Table 4). This can be due to various factors, such an observation was made by us with kits from other manufactures too¹⁷, and these kits are being successfully used for DNA genotyping with little or no discomfort.

This invasion resulted due to the shortening of the allelic bin at the D1S1656 locus. The allelic ladder of SF 25 STR reveals the allelic range from 11 to 19.3 at D1S1656 whereas the samples with conflicting profiles possess allelic designation of 8 or 9 when genotyped with PowerPlex® Fusion 6C system (Fig. 13). For the locus D1S1656, allelic bin range of the most commonly used multiplex kits are shown in Table 4.

Table 4: Allelic ladder bin range of the locus D1S1656 in the most commonly used multiplex kits								
Locus	SF25™ PCR amplification kit	GlobalFiler™	VeriFiler™ Plus PCR Amplification Kit	PowerPlex® 21 system	PowerPlex® Fusion 6C system	VersaPlex™	PowerPlex® Fusion 5C system	Sure ID PanGlob Human I Identific Kit
D1S1656	11,12,13,14,15,16,17,17.3,18.3,19.3	9, 10, 11, 12, 13, 14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19.3, 20.3	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19.3, 20.3	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19.3, 20.3	11-17, 18.3

Inhibitor tolerance:

SF25™ PCR amplification multiplex kit was tested for inhibitor tolerance from most commonly encountered inhibitors for DNA typing viz., hematin, humic acid, tannic acid, and EDTA. The stock of inhibitors was diluted into various concentrations for the desired study. Concentrations of Hematin used were as 500mM, 750mM, and 1000mM; For humic the concentrations used were 100ng/μL, 200ng/μL, and 300ng/μL; tannic acid as 500ng/μL, 750ng/μL, and 1000ng/μL; and

EDTA concentrations used were 750mM, 1000mM, and 1250mM. For accuracy and consistency, the effect of the inhibitors was tested in triplicates. PCR amplification was tested for 25 µL of total reaction volume, as per the recommendations in the protocol by the manufacturer.

With hematin 100%, 38% and 14% allelic calls were observed at the concentrations of 500mM, 750mM, and 1000mM respectively. With humic acid, 100%, 56%, and 24% allelic calls were observed at the concentrations of 100ng/µL, 200ng/µL, and 300ng/µL respectively. With tannic acid 98%, 69%, and 49% allelic calls were observed at the concentrations of 500ng/µL, 750ng/µL, and 1000ng/µL respectively. With EDTA 99%, 37% and 10% alleles were called at the concentrations of 750mM, 1000mM, and 1250mM respectively (Fig.14). Thus, the small variation in the concentration of inhibitors can lead to noticeable differences in PCR amplification, which is observed consistently with various other PCR reaction systems too. The presently observed inhibitor tolerance is very similar to the one reported by Ensenberger, M. G. *et al.* (2016) in their study¹⁸.

Conclusion:

SF25™ PCR amplification kit which includes 25 STR loci was developed for forensic DNA application and anthropological studies. This kit was evaluated for sensitivity, mixer interpretation, concordance, sample quantity, and inhibitor tolerance. The majority of our findings have demonstrated that the kit has performed as expected concerning sensitivity where a full profile was observed at 125pg of sample concentration. With regards to the mixture interpretation, the expected outcome was observed as almost 100% accurate profiles were obtained when the mixture ratios were 9:1 Male: Female ratios respectively. With respect to the reaction volumes, full profiles were obtained when the reaction volumes were kept at 25µL, 12µL, or 6µL wherein the template DNA added was 1ng at 125pg concentration, this suggests that the SF25™ PCR amplification kit reagents are very effective even with compromised sample volumes, as opposed to what has been suggested at 25µL, as being the needed volume for obtaining full profiles. The Inhibitor tolerances study demonstrated that the SF25™ PCR amplification kit reagents withstand minor concentrations of the inhibitors that are commonly encountered *viz* hematin, humic acid, tannic acid, and EDTA, it has been observed consistently that, elevation of these inhibitors could affect the performance of the kit, as observed for many such other kits. It becomes imperative that the DNA purifications should be such that, best can be done in minimizing these inhibitors, to extract the best profile while using these kits. With credible finding concerning high sensitivity at the low quantity of input DNA, and high inhibitor tolerance it has also been observed that the kit is not devoid of minor discrepancies, in some of the forensic samples run, in roughly about 12.05% DNA profiles, there has been a misrepresentation of triallelic pattern, this also can be viewed as a discrepant heterozygous pattern at D16S539 locus. This in few of these cases has resulted in a homozygous condition at locus D1S1656 due to invasion of alleles at D1S1656 locus to adjacent D16S539 locus, primarily as noticed, due to shortening of the allelic bin at D1S1656 locus. This study has successfully demonstrated the efficacy of the SF25™ PCR amplification kit, by covering all aspects that establish its acceptable use in DNA genotyping for forensic purposes this study validates its use in forensic laboratories. However, as with any reagent kit that is used for human Identification and DNA profiling, this kit has to be used as per the standard operating procedures set and followed by individual laboratories.

Declarations:

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Author Contributions

P.S. conceptualized the study and did genotyping. R.K. did the statistical analysis and wrote the manuscript. P.K., A.K., K.V.V.S and M.R. helped in experimental work. V.B, S.R., S.D. helped in preparing the manuscript. H.R.D, K.K., A.S., and G.C. reviewed the manuscript. All the authors reviewed and approved the manuscript.

Additional Information

Competing financial interests: The authors declare no competing financial interest.

Conflict of interest: None to declare.

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Figures

Figure 1

The locus and fluorescent dye label assignment for SF25™ PCR amplification kit.

Figure 2

Representative SF25™ Electropherogram obtained from 125 pg DNA using 6 µL PCR reaction volume.

25µL PCR reaction volume		1ng	500 pg	250 pg	125 pg	62.5 pg	31.25 pg	15.62 pg
SF25™ PCR amplification kit	D3S1358							
	D13S317							
	D7S820							
	D16S539							
	D1S1656							
	Penta E							
	TPOX							
	TH01							
	D2S1338							
	CSF1PO							
	Penta D							
	D19S433							
	vWA							
	D21S11							
	D18S51							
	D6S1043							
	Amelogenin							
	D8S1179							
	D5S818							
	D12S391							
	FGA							
	D22S1045							
	Yindel							
	D2S441							
	D10S1248							

Figure 3

Sensitivity of SF25™ PCR amplification kit at 25 µLPCR reaction volume.

12.5 µL PCR reaction volume		1ng	500 pg	250 pg	125 pg	62.5 pg	31.25 pg	15.62 pg
SF25™ PCR amplification kit	D3S1358							
	D13S317							
	D7S820							
	D16S539							
	D1S1656							
	Penta E							
	TPOX							
	TH01							
	D2S1338							
	CSF1PO							
	Penta D							
	D19S433							
	vWA							
	D21S11							
	D18S51							
	D6S1043							
	Amelogenin							
	D8S1179							
	D5S818							
	D12S391							
	FGA							
	D22S1045							
	Yindel							
	D2S441							
	D10S1248							

Figure 4

Sensitivity of SF25™ PCR amplification kit at 12.5 µLPCR reaction volume.

6 µL PCR reaction volume		1ng	500 pg	250 pg	125 pg	62.5 pg	31.25 pg	15.62 pg
SF25™ PCR amplification kit	D3S1358							
	D13S317							
	D7S820							
	D16S539							
	D1S1656							
	Penta E							
	TPOX							
	TH01							
	D2S1338							
	CSF1PO							
	Penta D							
	D19S433							
	vWA							
	D21S11							
	D18S51							
	D6S1043							
	Amelogenin							
	D8S1179							
	D5S818							
	D12S391							
	FGA							
	D22S1045							
	Yindel							
	D2S441							
	D10S1248							

Figure 5

Sensitivity of SF25™ PCR amplification kit at 6 µLPCR reaction volume.

Figure 6

Peak height ratio and percentage of alleles called at different DNA input concentration.

Figure 7

Effect of different PCR cycle numbers on mean peak height

Figure 8

Locus wise peak height comparison at PCR cycle number 28, 30 and 32.

Figure 9

SF25TM PCR amplification kit performance on different PCR thermal cyclers. Different amounts of control DNA 2800M were used as indicated in the figure. Each sample was run in triplicate. A standard 29 cycle protocol was used.

Figure 10

Intracolor peak height ratios for each dye channel.

Figure 11

Percentage of alleles called for various Male: Female DNA dilution ratio

Figure 12

concordance study of the SF25TM PCR amplification kit and PowerPlex® Fusion 6C system.

Figure 13

(A): False triallelic pattern at locus D16S539 (B): false heterozygous pattern at locus D16S539.

Figure 14

Inhibitors tolerance study for most commonly encountered inhibitors in DNA typing.