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Original research article

Comparisons of DNA Analysis using Qiagen® Investigator® 24plex QS Kit and AmpFlSTR® Identifiler® Plus PCR Amplification Kit

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ABSTRACT

At present, DNA test kits play a major role in DNA analysis, especially for forensic DNA. AmpFlSTR® Identifiler® Plus PCR amplification kit, for example, is a reagent kit that is commonly used in most forensic laboratories in Thailand. However, this kit offers only 15 loci and amelogenin. Recently, Qiagen® Investigator® 24plex QS kit, a newly-launched product, has been developed with its expanded 22 polymorphic STR markers, the gender-specific amelogenin and the Quality Sensor (QS) to address the forensic challenges. This research aimed to determine the optimized conditions for using Qiagen® Investigator® 24plex QS kit and compare DNA profiles which were amplified by the use of Qiagen® Investigator® 24plex QS and AmpFlSTR® Identifiler® Plus PCR amplification. A total of 40 DNA samples were undertaken in this research including samples with high and low DNA concentration, samples with inhibitors, and degraded samples. The results showed the PCR reaction of 1.0 ng/µl with full and half volume reactions were the same allele percentage detection. In concentration between 0.5 and 0.0625 ng/µl, the half volume reaction provided the percentage of allele detection that was higher than the full volume reaction. For DNA profiles comparison, the percentage of allele detection and heterozygous peak height ratio (PHR) in both kits showed similar results. Conclusion: The Qiagen® Investigator® 24plex QS kit was a reliable identification for forensic DNA analysis.

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Keywords: Forensic science; Qiagen® investigator® 24plex QS; DNA analysis; Allele detection

1. Introduction

The development of forensic DNA analysis has greatly abetted the resolution of the criminal investigation in general. Since the beginnings of crime investigation, forensic identification of human samples collected from crime scenes has been an integral part of forensic investigation. The growing global population complicates this process as it increases the pool of DNA profiles to match. The AmpFlSTR® Identifiler® Plus PCR amplification kit that targets 15 short tandem repeats (STRs) and one amelogenin gender-determination was one of the commonly used DNA analyses in forensic laboratories [1]. The efficiency of this method has been limited because some locus may repeat in different individuals. At present, a new reagent kit (Qiagen® Investigator® 24plex QS kit) has been developed to analyze 22 polymorphic STRs with gender-specific markers (amelogenin) and Quality Sensor (QS1 and QS2) [2]. These STR markers were recommended by the CODIS (Combined DNA Index System) Core Loci Working Group, the European Network of Forensic Science Institutes (ENFSI), and the European DNA Profiling Group (EDNAP) [3-6].

This research aimed to determine the optimal conditions for using the Qiagen® Investigator® 24plex QS kit for DNA testing and to compare its DNA profiles with the AmpFlSTR® Identifiler® Plus PCR amplification kit.

2. Materials and Methods

2.1 Optimal conditions of qiagen® investigator® 24 plex QS kit testing

A buccal swab DNA sample that has been used as a standard reference DNA control 9948 that was provided with the kit was quantitated by using Quantifiler™ Human DNA Quantification kit (Applied Biosystems, USA) on a 7500 Real-Time PCR system and diluted into 5 dilutions: 1.0, 0.5, 0.25, 0.125 and 0.0625 ng/µl, respectively. Duplicate DNA dilution samples were amplified with full (25 µl) and half (12.5 µl) volume reactions as shown in Table 1. The standard cycling protocol of PCR reaction has the following conditions: 3 cycles of 98°C for 30 sec; 64°C for 55 sec; and 72°C for 5 sec, followed by 27 cycles of 96°C for 10 sec; 61°C for 55 sec; and 72°C for 5 sec, followed by 68°C for 2 min and 60°C final extension for 2 min and the 10°C soak [2]. PCR products of each dilution were analyzed by using an ABI 3500 Genetic Analyzer.

Table 1. PCR master mix of full and half volume reactions.

Components	Full reaction (µl)	Half reaction (µl)
Fast Reaction Mix 2.0	7.5	3.75
Primer Mix	2.5	1.25
Nuclease-free water	5.0	2.50
Template DNA	10.0	5.00
Total	25.0	12.5

2.2 Comparison of DNA Profiles

2.2.1 DNA samples

All samples used in this study were chosen from previous forensic caseworks that had already been through DNA processing, including DNA extraction, DNA amplification (using AmpFlSTR® Identifiler® Plus PCR amplification kit), DNA fragment analysis (using GeneMapper ID-X v3.2.1 software, Applied Biosystems) and kept at -20°C. All DNA profiles from these samples were recorded in the database.

The 40 chosen DNA samples used in the experiment were as follows: 10 high DNA concentration samples, such as blood, semen and buccal swab; 10 low DNA concentration samples or DNA from the touch surface areas; 10 DNA samples with the inhibitor (nails and cigarette butts); and 10 degraded DNA samples from decomposed samples.

This study was reviewed and approved by The Ethical Review Sub-Committee Board for Human Research Involving Sciences, Thammasat University, No. 3 (031/2561) and Institute Biosafety Committee of Thammasat University.

2.2.2 DNA quantification

The control DNA sample and 40 DNA casework samples were measured using the following steps: 1 µl extracted DNA was added into 6.25 µl Quantifiler™ Human Primer Mix reagent and 5.25 µl Quantifiler™ PCR Reaction Mix reagent and then quantitated by Quantifiler™ Human DNA Quantification kit on 7500 Real-Time PCR system [7].

2.2.3 STRs amplification

The quantitated DNA from 40 casework samples were amplified using the Qiagen® Investigator® 24plex QS kit. A half volume reaction of 12.5 µl that contained 3.75 µl fast reaction mix 2.0, 1.25 µl primer mix, 7.5 µl nuclease-free water and DNA template was performed using GeneAmp® PCR System 9700 v3.12 with the following conditions: 3 cycles of 98°C for 30 sec; 64°C for 55 sec; and 72°C for 5 sec, 27 cycles of 96°C for 10 sec; 61°C for 55 sec; and 72°C for 5 sec, and 68°C for 2 min, 60°C for 2 min for the final extension and 10°C soaking [2].

2.2.4 DNA detection

Add 1 µl of PCR product into 12 µl Hi-Di Formamide and 0.5 µl of DNA size standard 24plex (BTO). The mixed solution was denatured at 95°C for 3 min. The denatured PCR solution was pipetted into a 96 well PCR plate. DNA electrophoresis was performed using the Applied Biosystems® 3500™ Genetic Analyzer.

All alleles in DNA profiles were identified using GeneMapper® ID-X v3.2.1 software (Applied Biosystems®) [2].

2.2.5 Statistical analysis

To determine the optimal conditions for using the Qiagen® Investigator® 24plex QS kit, allele percentage from both reaction volumes (full and half volumes) were analyzed using a Paired T-test.

In the evaluation and comparison of DNA profiles from AmpFlSTR® Identifiler® Plus amplification kit and Qiagen® Investigator® 24plex QS kit, the allele detection percentage and the heterozygous peak height ration (PHR) were statistically analyzed by the use of Wilcoxon signed-rank test. The statistically significant level was set at α=0.05.

3. Results and Discussion

3.1 Optimal conditions of qiagen® investigator® 24 plex QS kit

The percentage of allele detection from both full and half reaction volumes showed a 100% agreement when using 1.0 ng/µl. Interestingly, a higher allele detection percentage was observed when using DNA dilutions of 0.5, 0.25, 0.125 and 0.0625 ng/µl for the half PCR reaction volumes as compared to those of the full reaction volumes. However, the allele detection percentage from both half and full reaction volumes for 4 dilutions were significantly different (p=0.001) as shown in Fig. 1. The best optimal conditions for using Qiagen® Investigator® 24plex QS kit was observed when using 0.5 ng/µl DNA for the half-reaction volume.

A buccal-swabbed DNA sample which is routinely used as a control DNA in the forensic laboratory was also used in this study in order to establish the optimal conditions for using Qiagen® Investigator®

24plex QS. The quantity of buccal-swabbed DNA was standardized in accordance with the control DNA 9948 which is provided in the kit. In this study 5 dilutions were varied, namely 0.0625, 0.125, 0.25, 0.5 and 1.0 ng/µl. Kraemer *et al*. had similarly studied the sensitivity of this kit by using control DNA 9948 in which dilutions ranged from 1.0 down to 0.008 ng per reaction and reported a consistent 100% allele detection for 0.125 ng dilution [8]. This research had not only followed the manufacturer's protocol but also tested the half reaction volumes. Of the 5 dilutions, 0.5 ng/ µl exhibited the best concentration for the half PCR reaction volume. On this account, using half PCR reaction volume could be relatively cost-effective.

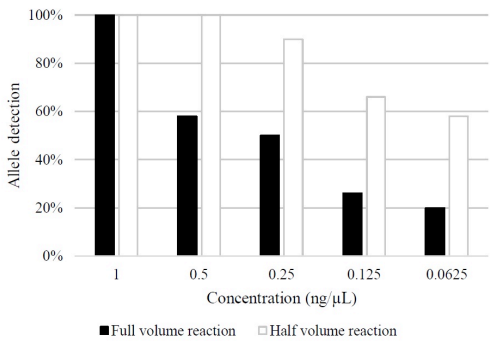

Fig. 1. Percentage allele detection of full and half volumes at 1, 0.5, 0.25, 0.125 and 0.0625 ng/µl.

3.2 Comparison of DNA profiles

3.2.1 Allele detection

The percentage values of allele detection obtained using Qiagen® Investigator® 24plex QS kit and AmpFlSTR® Identifiler® Plus PCR amplification kit were similar as shown in Fig. 2. Incidentally, sample no. 15 and 20 were not amplified with the AmpFlSTR® Identifiler® Plus PCR amplification kit. Sample no. 21, which may have a lot of inhibitors to block the amplification process, was not amplified in either kit. However, they were not significantly different (p=0.741).

3.2.2 Heterozygous peak height ratio (PHR)

Heterozygous Peak Height Ratio (PHR) of both kits were also similar as shown in Fig. 3. They were not significantly different (p=0.121). However, three samples (nos. 15, 20 and 33) showed only PHR results in the Qiagen® Investigator® 24plex QS kit. Three samples (nos.19, 21 and 32) did not show PHR because sample nos. 19 and 21 may have low DNA quality and many inhibitors, on the other hand, sample no. 32 may only have the homozygous allele.

In this study, higher quality DNA profiles including allele detection and PHR could be obtained from the Qiagen® Investigator® 24plex QS kit than from the AmpFlSTR® Identifiler® Plus PCR amplification kit. Moreover, most of the samples with inhibitors, low DNA concentration, and degraded DNA could be successfully amplified with the Qiagen® Investigator® 24plex QS kit. These results were similar to that of Tan JYY's that evaluated Qiagen® Investigator® 24plex QS and 3 other STR-PCR kits. Qiagen® Investigator® 24plex QS has been shown to tolerate inhibitors [9]. Another study related to the evaluation of 3 new STR amplification systems with 6-dye chemistry, namely the Qiagen® Investigator® 24plex QS kit from QIAGEN, the GlobalFiler™ PCR amplification kit from Applied Biosystems™ and the PowerPlex® Fusion 6c System from Promega, reported that the Qiagen® Investigator® 24plex QS kit showed a higher tolerance to common PCR inhibitors, including humic acid and tannic acid, and exhibited slightly higher sensitivity in the profiling of minor components in the DNA mixtures [10].

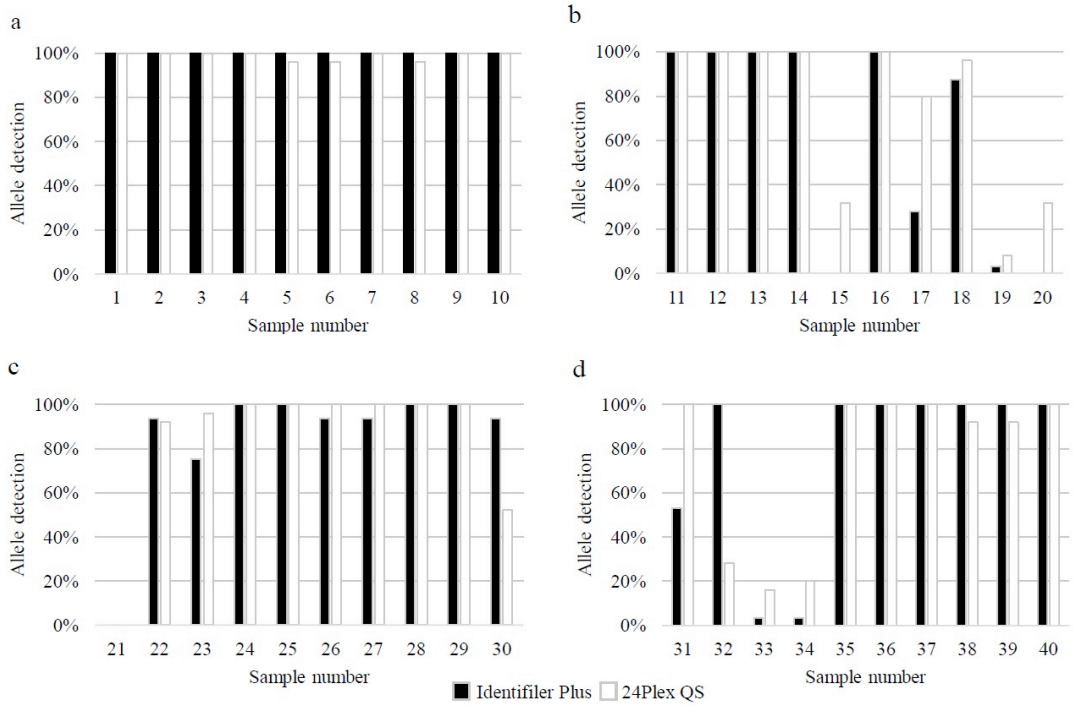

Fig. 2. Allele detection of Qiagen® Investigator® 24plex QS kit for casework samples; (a) high concentration samples, (b) low concentration samples, (c) samples with the inhibitor and (d) degraded DNA samples.

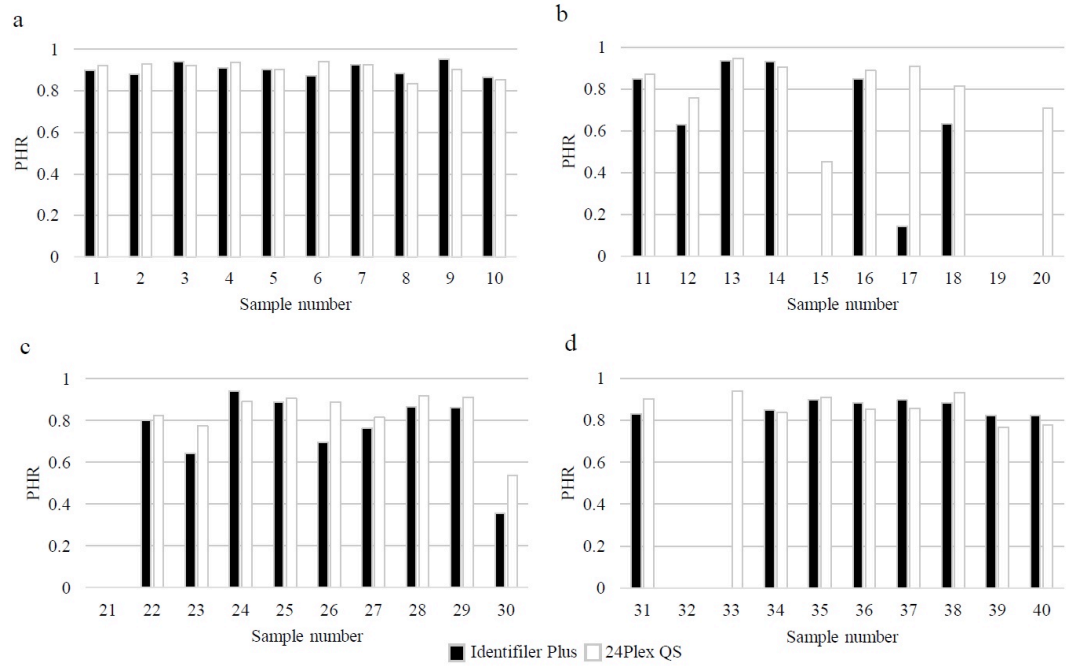

Fig. 3. Heterozygous peak height ratio (PHR) of Qiagen® Investigator® 24plex QS kit from casework samples; (a) high concentration samples, (b) low concentration samples, (c) samples with the inhibitor and (d) degraded DNA samples.

3.3 DNA profiles (Electropherogram)

Forty samples that have been amplified by using Qiagen® Investigator® 24plex QS kit were analyzed. All of the resultant DNA profiles from samples of high concentration samples represented complete profiles including QS1 and QS2 (Fig. 4a). Most of the low DNA concentration samples exhibited complete profiles. Some samples revealed no allele in some loci. However, alleles QS1 and QS2 were observable (Fig. 4b). The samples with inhibitor showed incomplete profiles with a ski-slope pattern. The peak height of QS1 and QS2 were similar (Fig. 4c). The degraded DNA samples showed an unclear ski-slope pattern, while QS1 and QS2 also showed a rather similar peak height (Fig. 4d).

The Qiagen® Investigator® 24plex QS kit can co-amplify 12 autosomal STRs markers, nine of which are not present in the AmpFlSTR® Identifiler® Plus PCR amplification kit [2, 11]. This kit has provided a set of efficient supplementary markers for human identification in the forensic laboratory.

4. Conclusion

The results indicated that the Qiagen® Investigator® 24plex QS kit could amplify 22 STR loci, amelogenin and the Quality Sensor (QS), while the AmpFlSTR® Identifiler® Plus PCR amplification kit could amplify 15 polymorphic STR markers and amelogenin. The best optimal used a DNA concentration of approximately 0.5 ng/μl by using half reaction volume. Since the power of discrimination depends on the number of STR loci detected, and some DNA test kits suffer from the forensic challenges resulting in incomplete profiles, the Qiagen® Investigator® 24plex QS kit that provides a higher number of STR loci and permits optimal results for a wide range of forensic samples namely inhibitors and degradation could be applicable to be used in forensic laboratories.

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Conflict of Interest

This research had no conflict of interest.

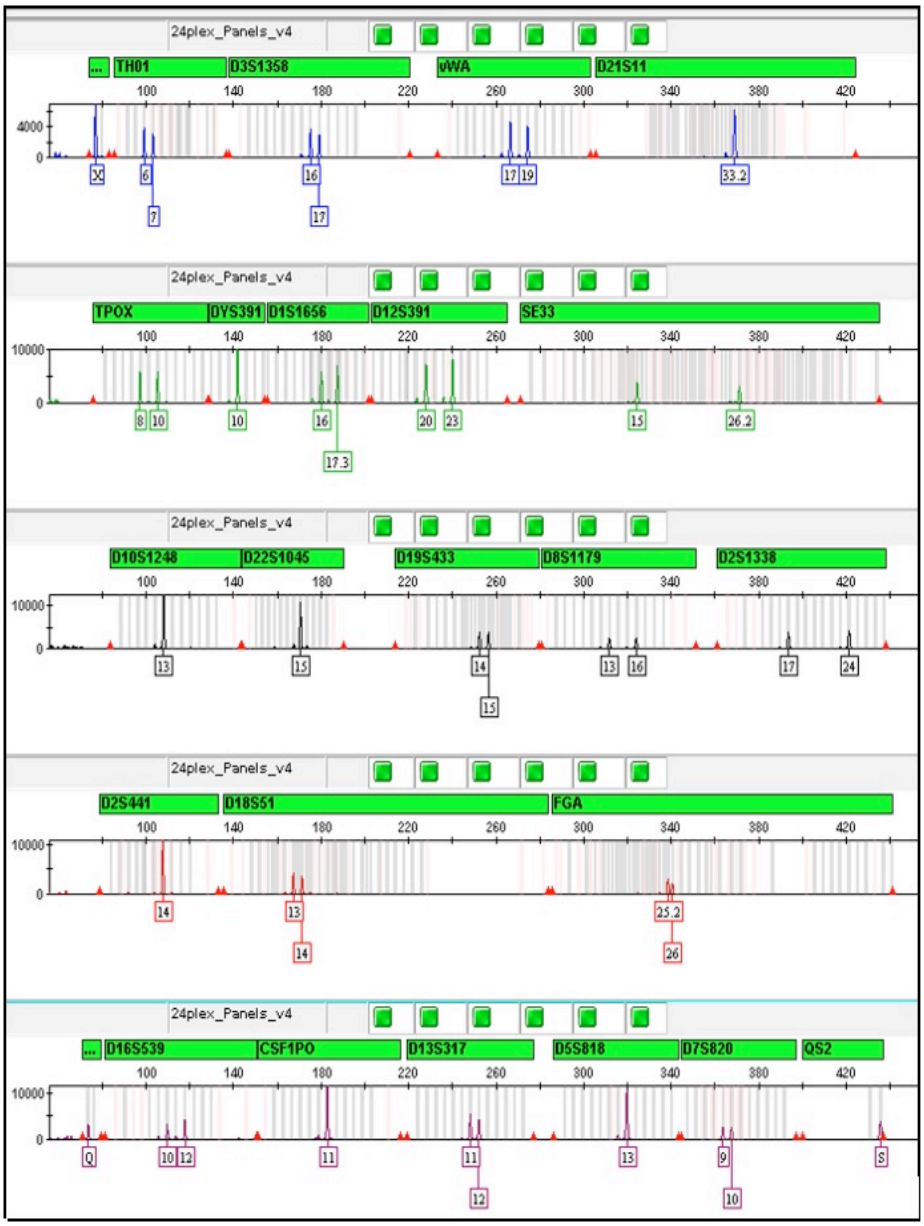

Fig. 4a. DNA profile: high concentration sample; no.3.

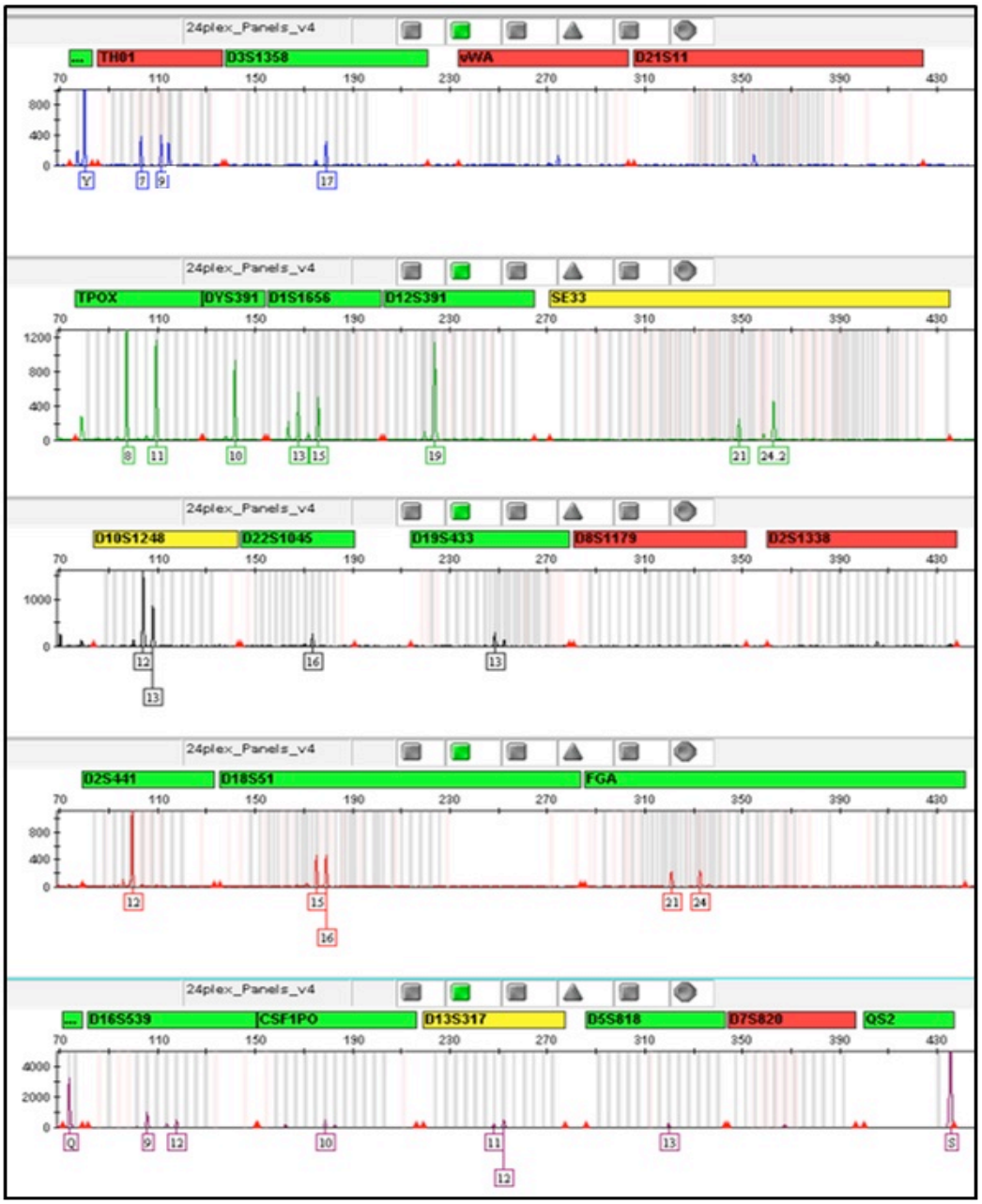

Fig. 4b. DNA profile: low concentration sample; no.17.

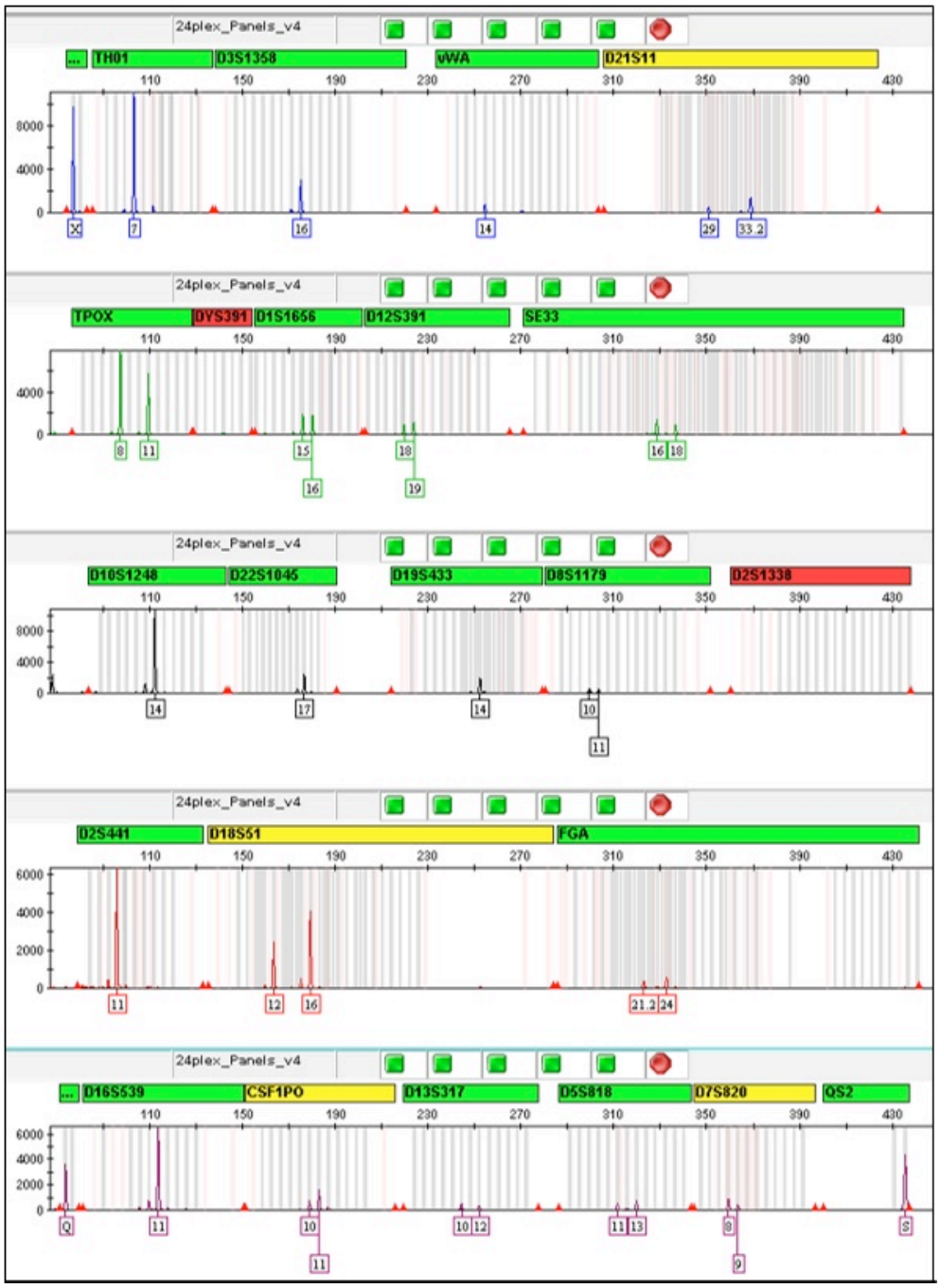

Fig. 4c. DNA profile: samples with the inhibitor; no.22.

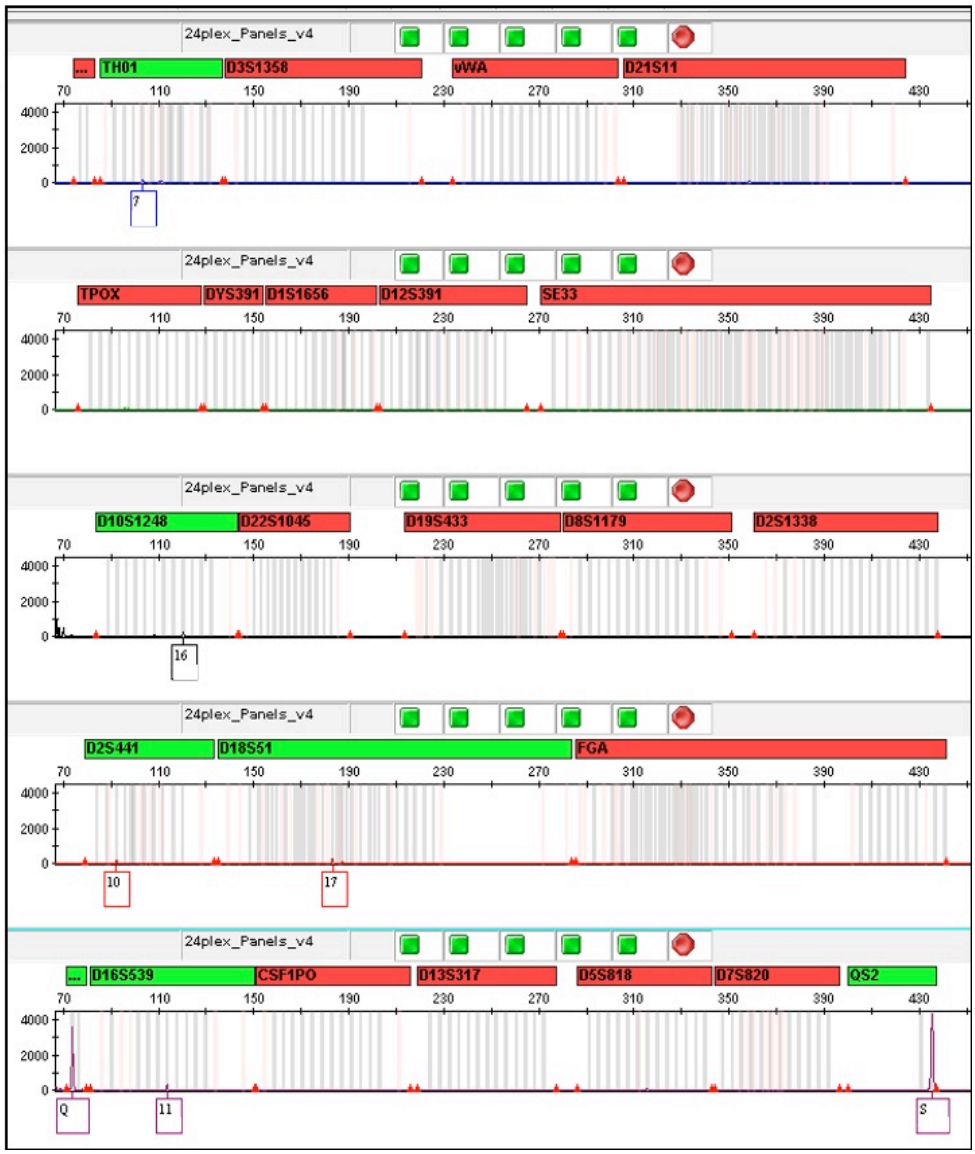

Fig. 4d. DNA profile: degraded DNA samples; no.32.

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