Validation Report December 2021

Developmental validation of the Investigator® Argus Y-28 QS Kit

The QIAGEN® Investigator Argus Y-28 QS Kit is intended for molecular biology applications in forensic, human identity, and paternity testing. The kit is used for multiplex PCR in forensic casework and was developed specifically for rapid and reliable generation of DNA profiles from blood, buccal swabs, and forensic stains.

The performance of the Investigator Argus Y-28 QS Kit was evaluated with regards to various sample types and conditions, commonly encountered in forensic and parentage laboratories.

The validation study was based on the recommendations of the European Network of Forensic Science Institutes (ENFSI) (1) and the Revised Validation Guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM) (2). The assay makes use of well-established methodologies for forensic DNA analysis. It co-amplifies 27 polymorphic Y-chromosomal STR markers and the Quality Sensor (QS) arranged in five fluorescent dye channels:

6-FAM™: DYS389-I, DYS391, DYS389-II, DYS533, DYS390, DYS627
BTG: DYS458, DYS393, DYS19, DYS437, DYS449
BTY: DYS460, DYS576, YGATAH4, DYS481, DYS448, DYS518
BTR2: DYS439, DYS549, DYS438, DYS456, DYS643
BTP: QS1, DYS570, DYS635, DYS385, DYS392, QS2

These genetic loci have been characterized in numerous studies by other laboratories (3–7). As a special feature, the Investigator Argus Y-28 QS Kit contains an internal PCR control (Quality Sensor QS1 and QS2), which provides helpful information about the efficiency of the PCR and the presence of PCR inhibitors. The internal

Quality Sensor is enclosed in the Primer Mix and amplified simultaneously with the polymorphic STR markers. For further information about the Quality Sensor and Y-STR locus information, please refer to the *Investigator Argus Y-28 QS Kit Handbook*.

Optimal reaction conditions were established, and the effects of variations in those conditions were assessed.

The effect of variations in buffer component concentrations is described using, as an example, $MgCl_2$ concentration (page 4). The robustness of the assay, regarding variations in PCR cycling conditions and a range of different thermal cyclers, was investigated (pages 4–9). The effects of using increased or decreased PCR cycle numbers were analyzed (page 6). Sensitivity was addressed by amplifying DNA of known concentration, in a range typically encountered in forensic casework analysis (page 10). Mixtures have been evaluated with regard to the ability to detect minor contributors in male/male mixtures (page 21), and to determine the tolerance towards large amounts of human background DNA in male/female mixtures (page 4).

Specific issues that can arise during forensic casework were investigated, such as the ability to obtain results from samples that have been subjected to adverse environmental conditions. This was tested using DNA spiked with several types of inhibitors (page 13) or degraded DNA (page 18). Cross-reactivity with non-human DNA (page 19) was also assessed. Reference samples have been tested for compatibility with direct amplification (page 26). The reproducibility of the results was verified (page 28).

Results of developmental validation

The validation study was performed at the QIAGEN R&D department. All of the electropherograms shown were generated on an Applied Biosystems® 3500™ Genetic Analyzer, or Applied Biosystems 3500™ XL Genetic Analyzer. The standard conditions specified in the *Investigator Argus Y-28 QS Kit Handbook* were used for electrophoresis. Unless stated otherwise, an Applied Biosystems Veriti® 96-Well Thermal Cycler, or a QIAGEN QIAamplifier 96 instrument was used for amplification. Data were analyzed using Applied Biosystems GeneMapper ID-X software, v1.5.

Reaction conditions

Reaction conditions were established for an optimal performance in terms of sensitivity, specificity, and reproducibility. This required the optimization of all critical buffer components. The final composition of the Fast Reaction Mix 3.0 is a robust buffer system that tolerates differences in the concentration of individual buffer components, without a decrease in overall amplification performance.

For example, various concentrations of $MgCl_2$, one of the critical buffer components, were added to a standard reaction with 500 pg control DNA 9948 (Figure 1). The assay yielded robust results within a $MgCl_2$ concentration range of ±17% of the optimum.

Under these conditions, amplification of the STR markers was well balanced and no dropout or non-specific amplification occurred. Similar experiments were performed to evaluate optimal concentration for other buffer components, such as the hot-start DNA polymerase, dNTPs, and BSA (data not shown).

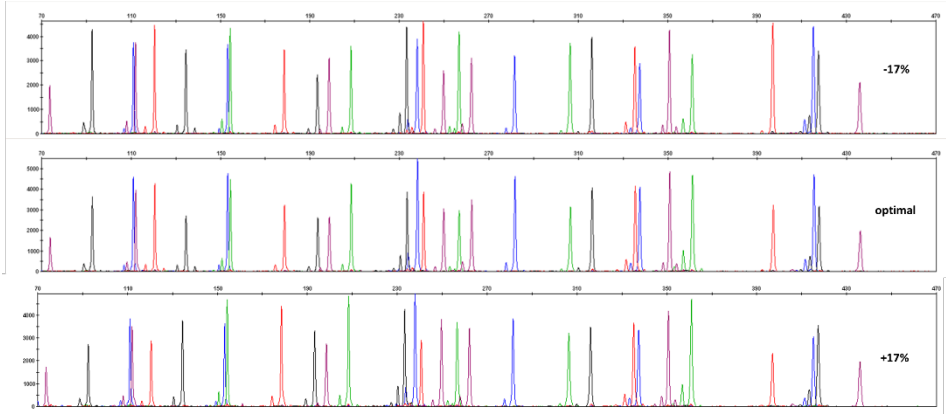


Figure 1. Influence of MgCl2 concentration. Fast Reaction Buffer 3.0 (FRM 3.0) was supplemented with different concentrations of MgCl2 matching the specification of production, or 17% higher and lower. Representative electropherograms of four replicates each are shown.

Effect of PCR annealing temperature variations

Specificity, sensitivity, and robustness are critical in forensic casework, all of which are affected by the annealing temperature (T_m). In Y-chromosomal STR analysis, an optimal annealing temperature is in particular important to avoid any amplification from autosomal or X-chromosomal sequences in samples having a high female background. Since the actual T_m may vary depending on cycler conditions, the assay was validated in a range surrounding the optimal T_m of the Investigator Argus Y-28 QS Kit reaction (30 cycles at 61.5°C).

Table 1. Recommended PCR cycling protocol.

Temperature	Time	Number of cycles
96°C	12 min	–
96°C 61.5°C 72°C	10 s 1 min 25 s 5 s	30 cycles
68°C	5 min	–
60°C	5 min	–
10°C	∞	–

Annealing temperatures between –3°C and +3°C around the optimal annealing temperature of 61.5°C were applied to the amplification of 500 pg control DNA 9948 (Figure 2). PCR was performed on a QIAamplifier 96 instrument. Reactions using annealing temperatures between –3°C and +1.1°C resulted in full profiles and good inter-locus balance. No non-specific PCR products were observed. Annealing temperatures significantly below the recommended temperature increase the risk of unspecific amplification from high levels of female background DNA.

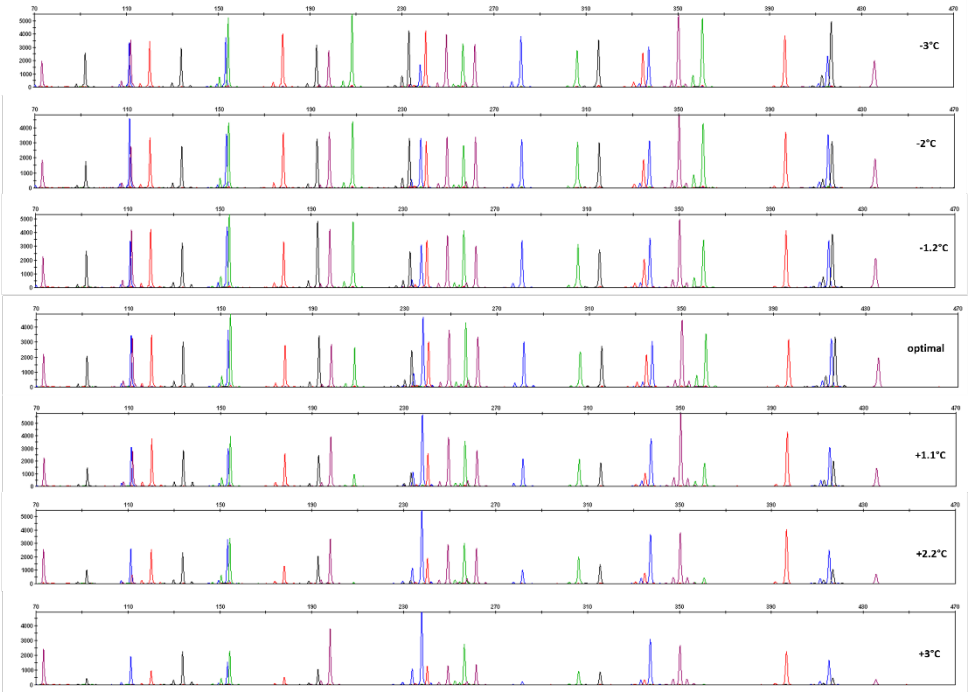


Figure 2. Effect of variations in the PCR annealing temperature on the amplification of the Y-STR markers. Under standard conditions (30 cycles), 500 pg control DNA 9948 was amplified on a QIAamplifier 96 instrument using an annealing gradient of 58.8–64.5°C. Representative electropherograms of triplicates are each shown.

Amplifications containing 3 µg of female DNA still show numerous products across all dyes, if the annealing temperature is 58.5°C, instead of 61.5°C. These amplification products gradually disappear when increasing the annealing temperature (Figure 3). The most prominent female artifacts observed in this study are located in the BTY and BTRII channel. BTY: 137 bp within DYS576, and 268 bp in the off marker range between DYS481 and DYS448. BTRII: 288 bp in the off marker range between DYS438 and DYS456, 352 bp within DYS456, and 369 bp within DYS643. In order to ensure optimal performance of the assay, we strongly recommend regular calibration of thermal cyclers.

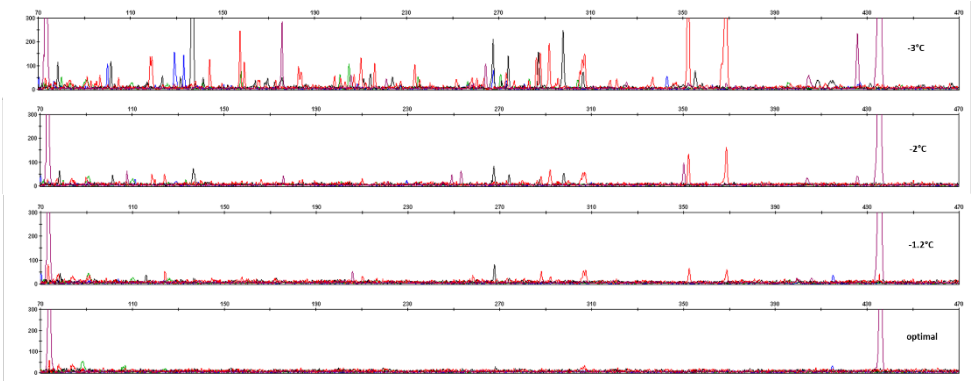


Figure 3. Impact of the PCR annealing temperature on the robustness towards female DNA. For analysis, 3 µg of DNA extracted from a blood sample of a female donor was amplified on a QIAamplifier 96. Representative electropherograms for reactions using annealing temperatures between 58.5°C and 61.5°C are shown. Electropherograms are scaled to 300 RFU.

Effect of different cycle numbers

PCR cycle numbers can be altered to adapt the reaction conditions to varying DNA template concentrations. Cycle numbers can be either increased to enhance amplification signals when working with low-copy-number DNA, or decreased to avoid overshooting profiles, when the DNA sample is abundant (e.g., for direct amplification of database samples).

Cycle number was increased to 31 for a dilution series of control DNA 9948, and the numbers of called alleles, as well as peak heights, were compared to a standard 30-cycle protocol (Figure 4). As expected, signal intensities of amplified products approximately double with the additional cycle. The increase in signal heights was accompanied by a moderate increase in alleles detected at a threshold of 50 RFU for template amounts below 125 pg (Figure 4). Note that the sensitivity of the capillary electrophoresis instrument, and the setting of the detection threshold strongly influence the outcome of an assessment of increased cycle numbers. Therefore, such protocol adaptations must be evaluated by individual laboratories using their instrumentation and analysis. Adapting the injection time of the capillary electrophoresis instrument or using post-PCR purification (e.g., the QIAGEN MinElute® PCR Purification Kit) provides alternative methods for increasing sensitivity.

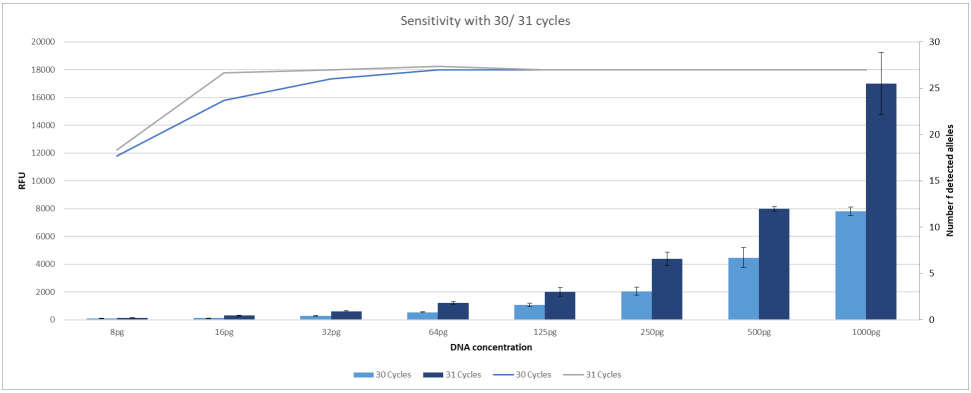


Figure 4. Effect of increased cycle numbers on mean peak height and number of called alleles. Different amounts of control DNA 9948 were used as template, as indicated in the figure. Samples were run in triplicates and average numbers of detected alleles per profile (indicated above the bars; 27 alleles expected in a full profile) and their peak heights were calculated. 50 RFU was used as a threshold for detection.

In particular, direct amplification from reference samples, such as blood or saliva on FTA cards, or buccal swab lysates, requires the evaluation of the optimal number of PCR cycles. The cycling protocols provided in the handbook should give a good starting point for the evaluation, but due to variations in sample materials (e.g. caused by sampling), cycle numbers may need to be increased or decreased to ensure optimal first-pass rates. Here, we used buccal swabs from 11 donors to create lysates for direct amplification with the Investigator

GO! Lysis Buffer. PCR was performed using 26, 27, and 28 cycles. Again, as expected, peak heights approximately doubled with each cycle added. Due to sampling and donor dependent differences, average signal heights differed by more than a factor 10 between the highest and the lowest sample (Figure 5). When amplified with 26 cycles, the weakest sample (donor 9) showed several alleles dropping below the analytical threshold of 200 RFU used in this experiment. When amplified with 28 cycles, the strongest sample (donor 3) on the other hand started to show pull up issues. Overall, 27 cycles provided the best data quality on this set of samples. The Quality Sensor has been designed to only show minimal response to changes in cycle numbers in a range from 25 to 31 cycles. This is illustrated in Figure 6, showing QS signals obtained in the direct amplifications from swabs.

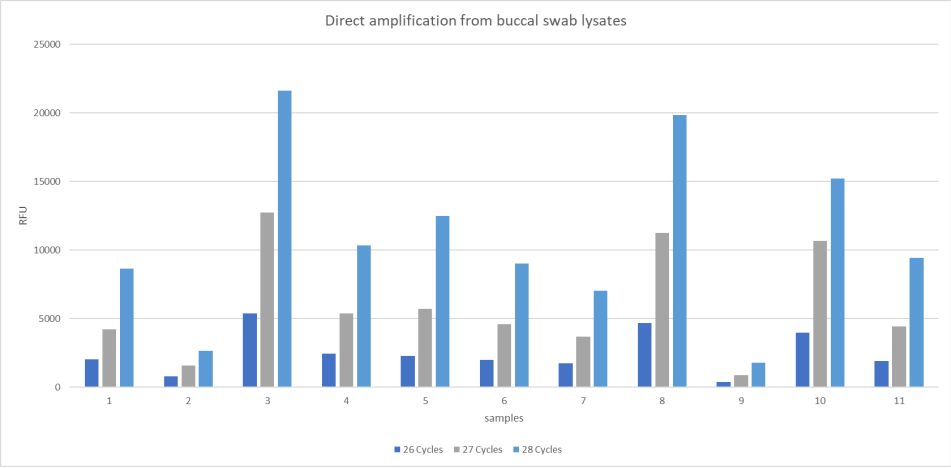


Figure 5. Effect of varying cycle numbers on mean peak heights in direct amplification of buccal swab lysates. Using 26, 27, or 28 PCR cycles, swab lysates rom 11 different donors were subjected to direct amplification. Samples were run in triplicates and average peak heights calculated.

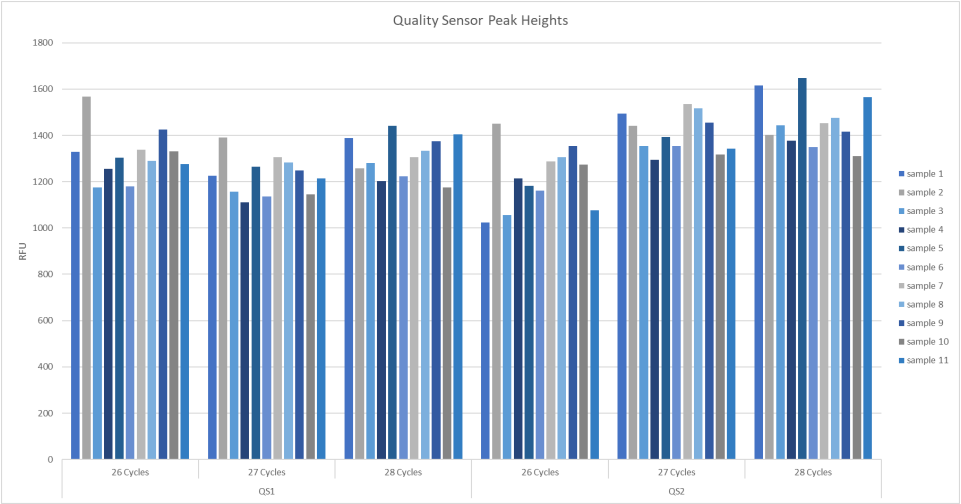


Figure 6. Quality Sensor QS1 and QS2 peak heights using 26–28 cycles. The Quality Sensor shows constant signal values.

Effect of different cycler types

Several PCR thermal cyclers were tested with the Investigator Argus Y-28 QS Kit, to demonstrate kit robustness across different instruments (Figure 7). As a PCR template, a dilution series of control DNA 9948 from 2 ng to 32 pg was used. The reaction took place under standard cycling conditions (see Table 1) and was performed with the following thermal cyclers.

- QIAGEN QIAamplifier 96
- Applied Biosystems GeneAmp® PCR System 9700 with Gold-plated Silver 96-Well Block
- Applied Biosystems Veriti 96-Well Thermal Cycler
- Eppendorf® Mastercycler® ep
- Bio-Rad C1000 Touch

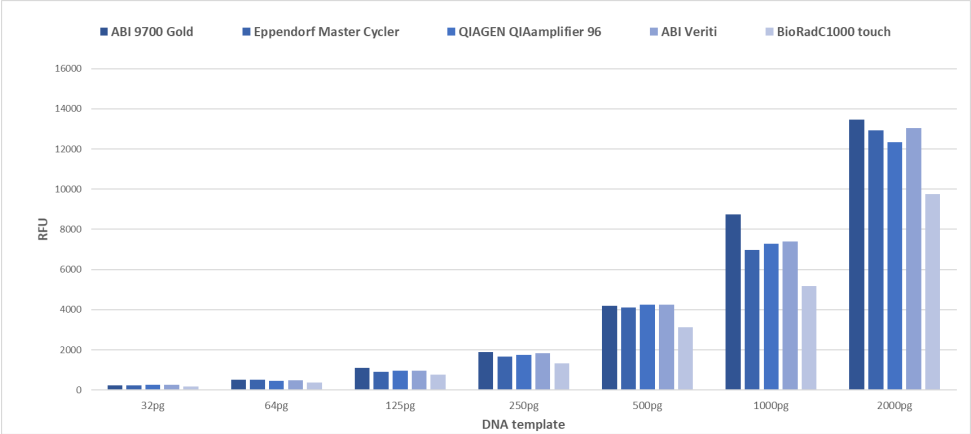


Figure 7. Performance on different PCR thermal cyclers. Different amounts of control DNA 9948 were used, as indicated in the figure. Each sample was run in triplicates. A standard 30-cycle protocol was used. Average peak heights across all markers are shown.

Comparable mean peak heights were obtained for all of the tested PCR cyclers. No significant differences in intra- and inter-locus balance or non-specific amplification were observed on any of the thermal cyclers.

Sensitivity

The Investigator Argus Y-28 QS Kit is designed to work robustly over a range of DNA quantities. The recommended amount of input DNA to yield good quality STR profiles is 500 pg, based on real-time PCR quantification of human DNA, for example, using the QIAGEN Investigator Quantiplex® Pro Kit. In particular, for heavily degraded DNA, the use of increased template amounts may improve results (see page 18).

Control DNA 9948 was serially diluted from 1 ng to 8 pg per reaction. Full profiles (27 PCR products) were consistently obtained down to 64 pg, using the standard conditions specified in the *Investigator Argus Y-28 QS Kit Handbook*. Occasional allele dropouts were found due to stochastic effects when ≤32 pg DNA was used as template. As expected, the number of dropouts increases with decreasing DNA concentration. See "Effect of different cycle numbers" and Figure 4 for expected numbers of detected alleles with low-copy-number samples.

Issues with very high amounts of DNA

Although DNA template amounts of >1 ng do not cause issues during PCR amplification, these samples can still result in inaccurate or unusable data. Depending on the instrumentation and settings used for capillary electrophoresis, fluorescence intensity may go off the scale. Due to the lack of homozygous alleles, there is in general better control over peak heights in Y-STR analysis, than there is in autosomal STR analysis.

"Off scale" peaks are often accompanied by "pull up" peaks. Furthermore, higher stutter-peak height ratios may be observed, as the signal from the main peak becomes saturated. Finally, "split peaks" may occur as a result of incomplete +A nucleotide addition.

Poor STR profiles resulting from high DNA concentration can be improved by re-amplifying a sample using less template DNA.

Issues with very low amounts of DNA

Amplification of less than ~100 pg DNA may lead to incomplete profiles, lacking one or more alleles. Furthermore, low allele copy numbers in the PCR can result in an unbalanced amplification of the alleles due to stochastic fluctuation.

These effects can be handled by either performing multiple amplifications of the same sample to create a consensus profile, or by adding the maximum volume of the DNA template to the PCR, in order to get the best possible result from a single reaction. For correct interpretation of samples having only small amounts of DNA, it is crucial to minimize background fluorescence, for example, caused by dye artifacts.

Figures 8 and 9 show examples of a no-template amplification. Peak heights of amplification products and the level of background noise depend on the instruments and settings of individual laboratories. We therefore recommend evaluating a suitable threshold for detection of alleles based on results obtained during an internal validation in the laboratory.

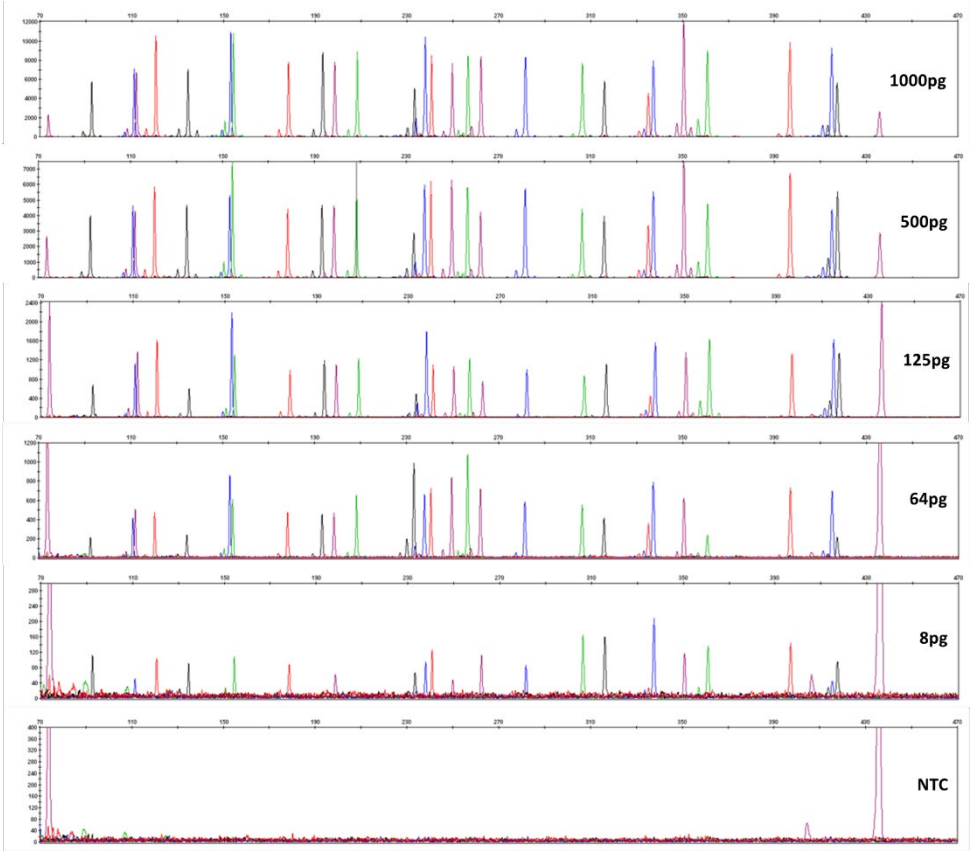


Figure 8. Sensitivity study. Serial dilutions of control DNA 9948 were analyzed. The amounts of DNA indicated were used as template for amplification. The lowest panels show a no-template (negative) control (NTC) to illustrate the expected level of background fluorescence. Y-axis scales were adjusted individually for the highest peak heights of the STR markers. **Note:** As the Quality Sensor (first and last purple peaks) is amplified with similar amplification efficiency in all experiments, independent of the sample template amounts, the QS peak heights are similar in all experiments.

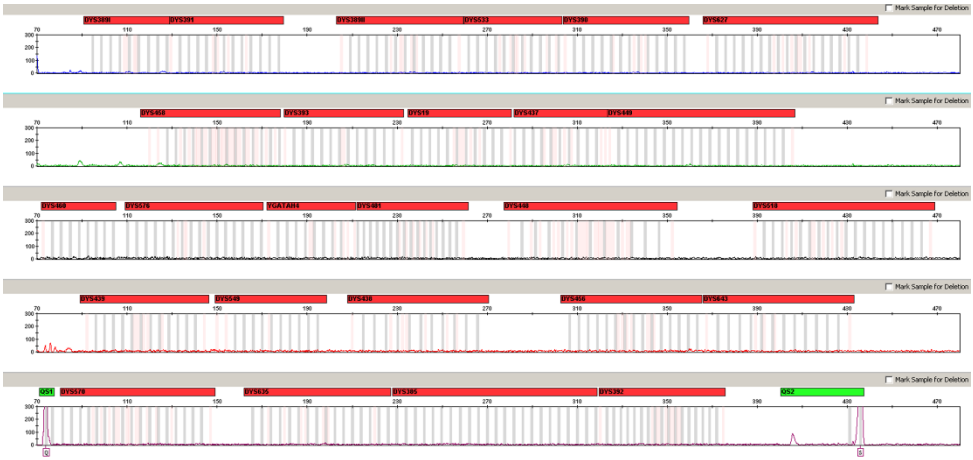


Figure 9. Background fluorescence. Example of a no-template (negative) control to illustrate the expected level of background fluorescence. For correct interpretation of samples having only small amounts of DNA, it is crucial to minimize background fluorescence.

Performance with simulated inhibition

If the DNA extraction from forensic casework samples is done using inappropriate methods, Investigator Argus Y-28 QS Kit performance may be compromised, although the reaction chemistry has been optimized for increased inhibitor tolerance. QIAGEN sample preparation technology is recommended for extraction, as it yields pure DNA free from inhibitors. Direct amplification from reference samples (e.g., blood on FTA cards) is another example for potential inhibition.

To test the robustness of the kit in the presence of inhibitors, assays were run in the presence of the following inhibitors, chosen to mimic challenging forensic sample types:

- Humic acid: A principal component of humic substances that has an inhibitory effect on PCR. It may be co-extracted from forensic samples collected from soil.
- Hematin: Formed by the oxidation of heme, the main component of blood. It has been identified as a PCR inhibitor in DNA samples extracted from bloodstains.

- Tannic acid: Typically present in leather. During extraction of casework evidence, tannic acid may be co-extracted with DNA.
- Indigo carmine: The color of blue denim (e.g., jeans).
- Collagen: The main protein compound of many tissues.
- Calcium: Released during lysis of bones.
- Ethanol: Potential carryover of the DNA extraction method.

Results are shown in Figures 10–16. Figure 16 shows an overview of Investigator Argus Y-28 QS Kit inhibitor resistance.

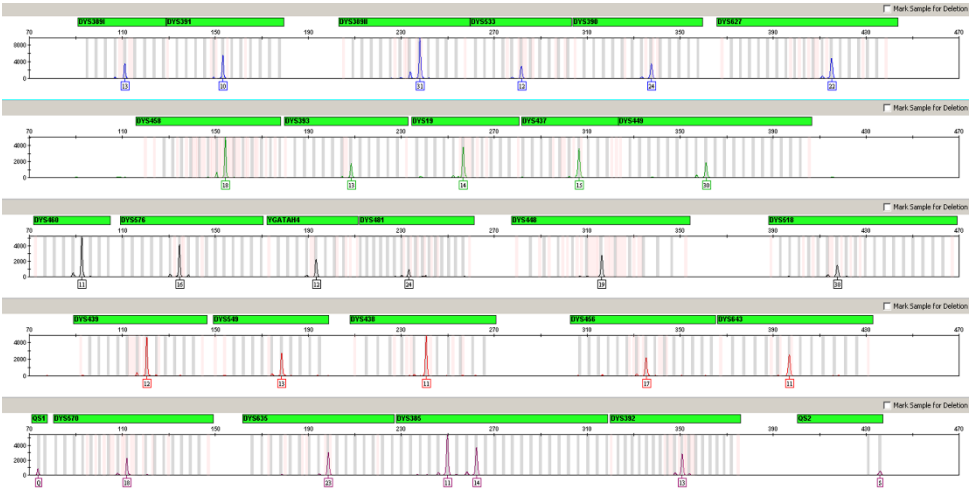


Figure 10. Electropherogram of 500 pg control DNA 9948 amplified in the presence of 500 µM hematin.

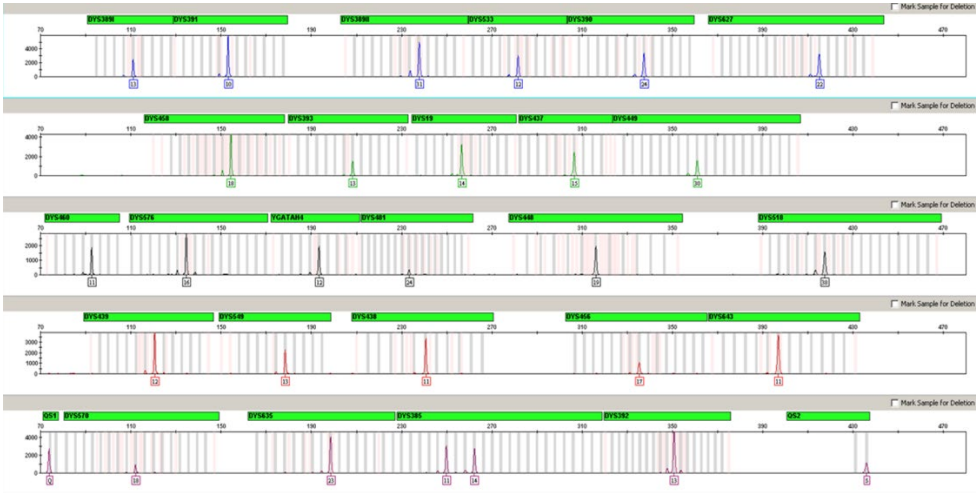


Figure 11. Electropherogram of 500 pg control DNA 9948 amplified in the presence of 150 ng/µl humic acid.

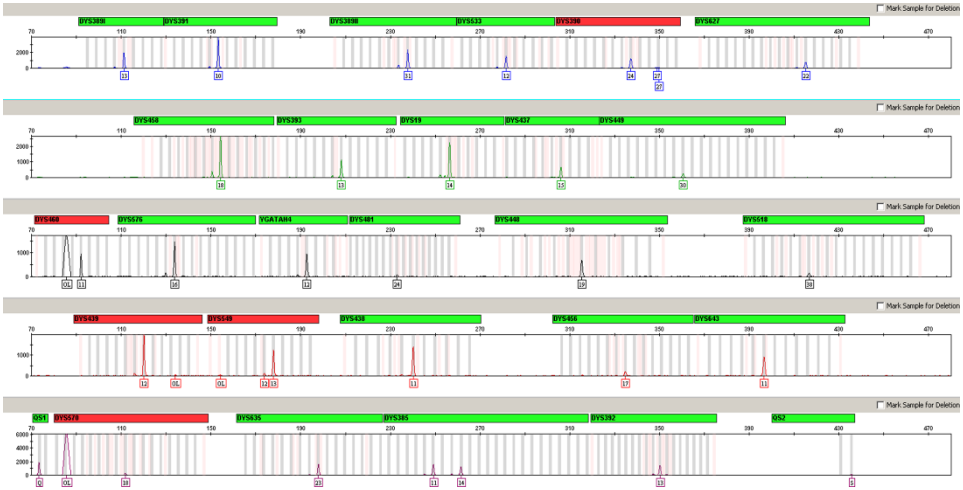


Figure 12. Electropherogram of 500 pg Control DNA 9948 amplified in the presence of 6 mM indigo carmine. Artifacts are due to fluorescent signals of indigo.

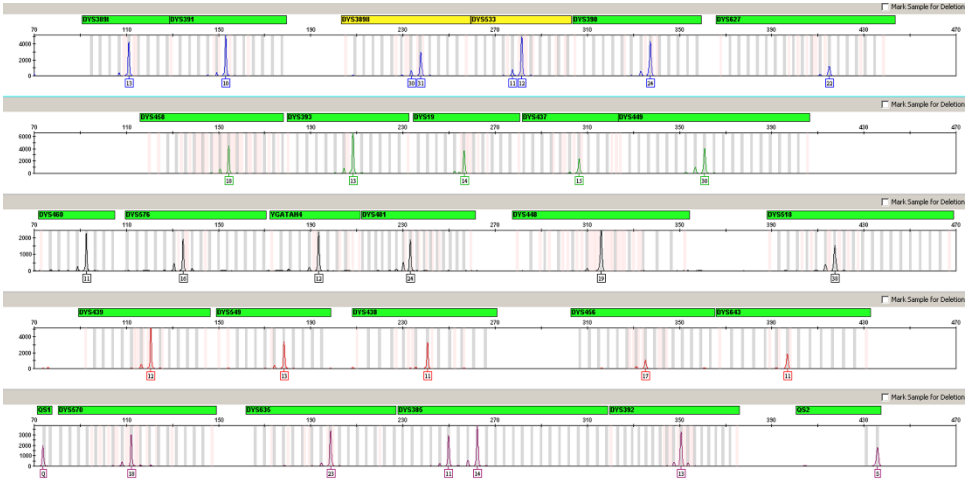


Figure 13. Electropherogram of 500 pg control DNA 9948 amplified in the presence of 2 mM calcium.

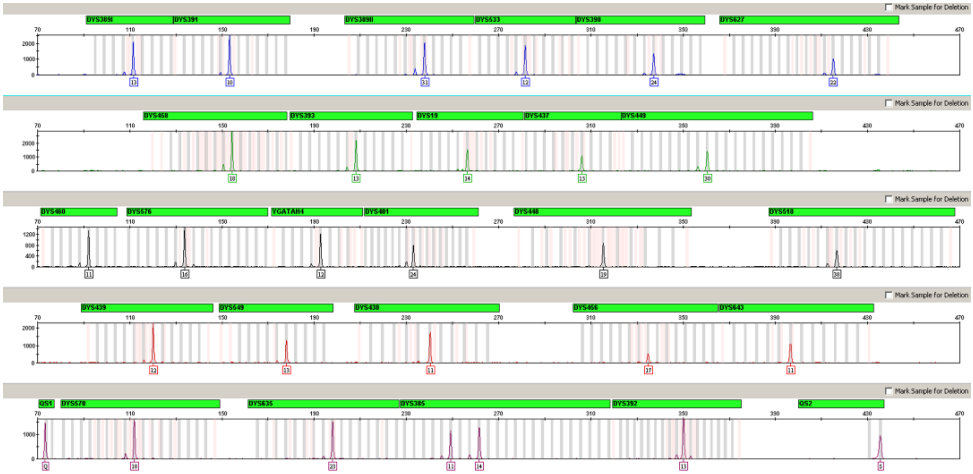


Figure 14. Electropherogram of 500 pg Control DNA 9948 amplified in the presence of 6000 ng/µl tannic acid.

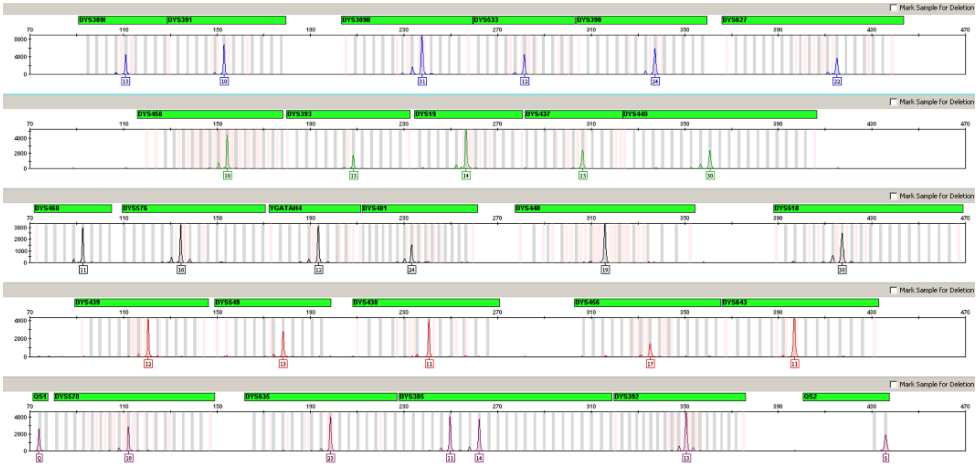


Figure 15. Electropherogram of 500 pg control DNA 9948 amplified in the presence of 2% ethanol.

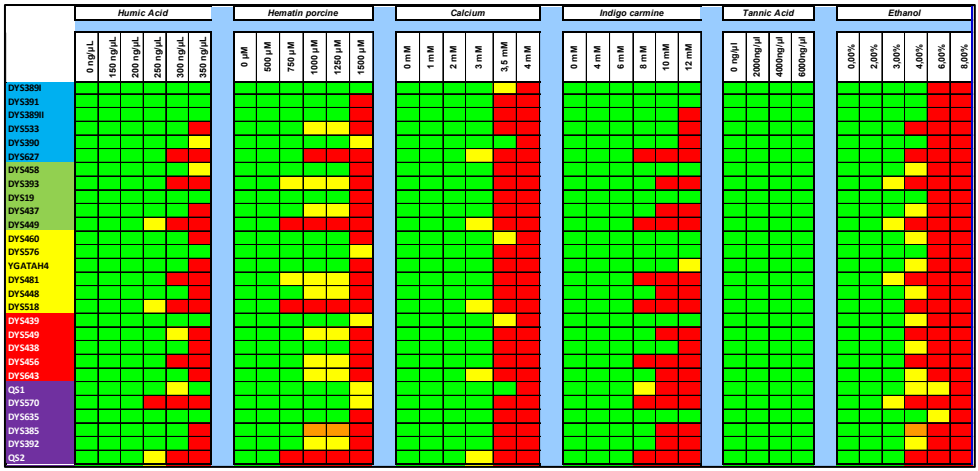


Figure 16. Overview of Investigator Argus Y-28 QS inhibitor resistance. The assay was tested for its robustness towards inhibitors (humic acid, hematin, tannic acid, indigo carmine, calcium, and ethanol). As template, 500 pg of control DNA 9948 were used and PCR was performed under standard conditions. As a threshold for allele calling, 50 RFU was used. Green: Consistently full profile. Yellow: 75% of expected PCR products detected. Orange: 50% of expected PCR products detected. Red: Less than 50% of expected PCR products detected.

Full profiles lacking any PCR artifacts, for example, split peaks, were obtained over a wide range of inhibitor concentrations. For most inhibitors, the degree of peak height reduction is correlated to the size of the PCR product, and dropouts are first observed for the markers having the largest amplicons. DYS481 and DYS570 are exceptions to this rule, as they have short-to-medium size amplicons, but are affected along with the large amplicon markers. The Quality Sensor QS2 responds equivalent to the most sensitive markers, confirming inhibition of the sample.

Note that the highest inhibitor concentrations used in this study will be found only rarely in casework samples, even if inappropriate sample extraction methods have been applied.

Stability with degraded DNA

Casework evidence has often been exposed to adverse environmental conditions, for example, UV light, humidity, or microbial growth. Human DNA extracted from these samples may therefore show varying degrees of degradation.

Artificially degraded male genomic DNA was used for validation. DNA was sheared using Adaptive Focused Acoustics™ (Covaris system, Covaris Inc., Woburn, MA, USA) to average fragment lengths of 500 bp, 300 bp, or 150 bp according to the manufacturer's instructions. 500 pg DNA was used as template for amplification. Full profiles were obtained with DNA of 300 bp and 500 bp average length. As expected, loci with larger amplicon sizes become affected as DNA degradation progresses (Figure 17). At an average fragment length of 150 bp, app. 60% of expected peaks were still detected using a threshold of 50 RFU. Increased amounts of template can be used to improve results for heavily degraded DNA.

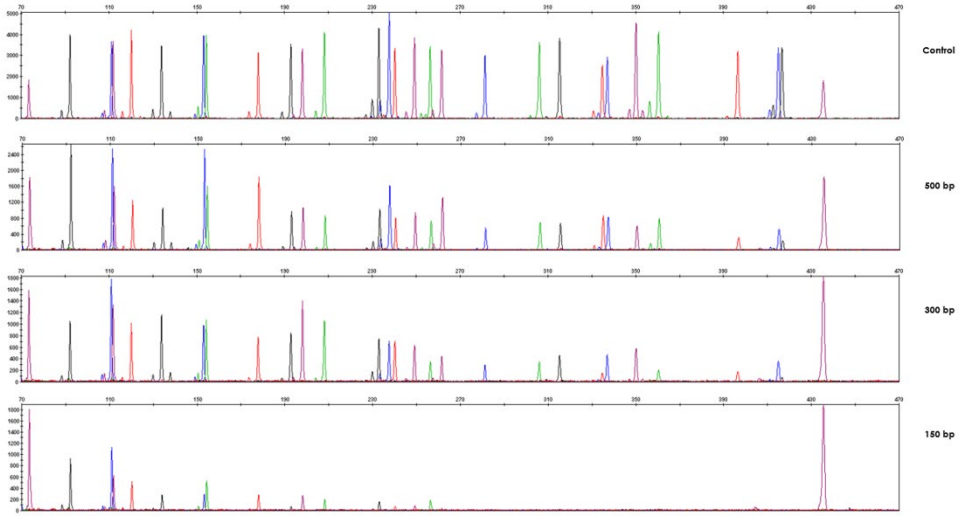


Figure 17. Degraded DNA. Male DNA was sheared to the defined average fragment length indicated. STR analysis of untreated control and degraded DNA samples. 500 pg template DNA was amplified in triplicates. Full profiles were obtained down to 300 bp average fragment length. Representative profiles are shown. **Note**: The Quality Sensor is not affected by the degraded DNA and was amplified with equal peak heights.

Species specificity

Non-human DNA can be present in forensic casework samples. It is critical that assays show no cross-reactivity between species. To verify Investigator Argus Y-28 QS Kit species specificity for human DNA, DNA from other species was tested following the standard assay protocol (Figure 18).

Besides common pets and farm animals, some primates were also tested. As expected for primates, amplification of some products is possible. Chimpanzees and bonobos give rise to several peaks within marker ranges in all channels, some of which match the size of human STR products.

None of the other animal DNAs did not show any cross reactivity with the Investigator Argus Y-28 QS Kit. Furthermore, following microbial organisms have been tested: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. No cross-reactivity was observed (data not shown).

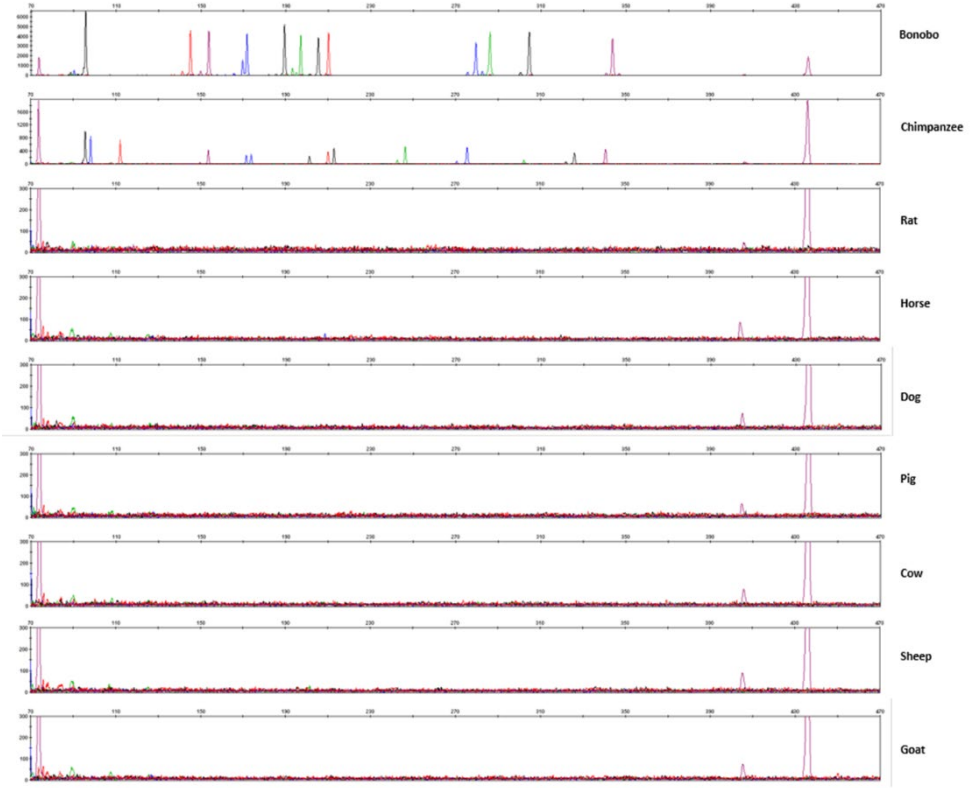


Figure 18. Representative results of the species specificity assessment. As template, 500 pg primate DNA and 2.5 ng DNA from all other species were used. The purple peak at app. 405 bp is a known CE artifact caused by the large Quality Sensor fragment.

Mixture studies

Evidence samples are frequently composed of more than one individual's DNA. For correct interpretation of results from mixtures, it is important to know the limit of the minor contributing component that still can be resolved.

Samples were created by mixing DNA QHID3 and control DNA 9948 and in ratios of 1:1, 3:1, 7:1, 10:1, 15:1 (see Table 3 for genotypes of mixed samples). The total amount of mixed DNA used in this study was 500 pg; a 15:1 mixture thus contains 33 pg of the minor component DNA and 467 pg of the major component (Table 2). The limit of detection of the minor component was determined by analyzing non-overlapping alleles of both DNAs. All expected alleles were found for minor components of 3:1 and 7:1 mixtures. 10:1 and 15:1 typically resulted in partial profiles of the minor component. An example for a 1:10 mixture is shown (Figure 19). Since these contain ≤50 pg of the minor component, the results are in concordance with the sensitivity for single-source samples reported here. In order to increase the sensitivity for the minor component, higher overall DNA amounts may be used if the amount of available DNA is not limited. See "Sensitivity", page 10, for general considerations.

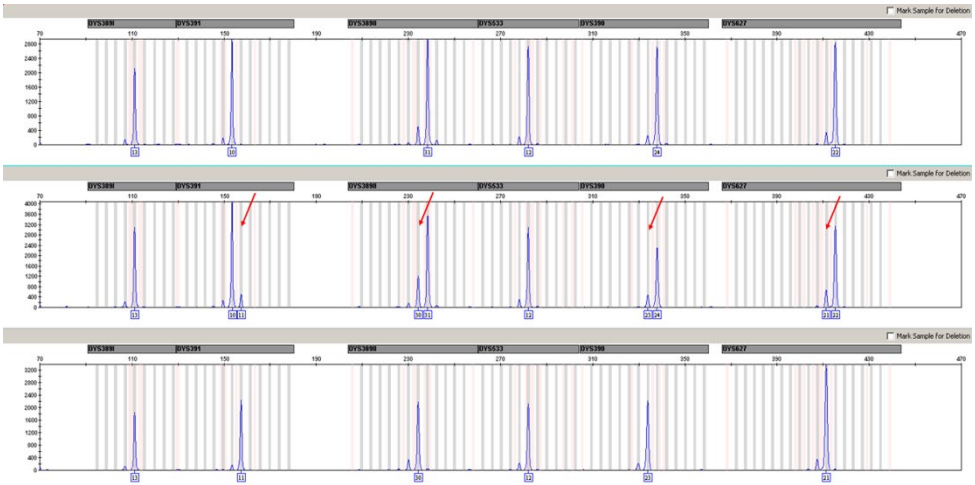
Table 2. Amounts of DNA template in mixtures.

Mixture ratio	Major component DNA QHID3	Minor component DNA 9948
1:1	250 pg	250 pg
3:1	375 pg	125 pg
7:1	430 pg	70 pg
10:1	450 pg	50 pg
15:1	467 pg	33 pg

Table 3. Genotypes of DNAs used for mixed samples.

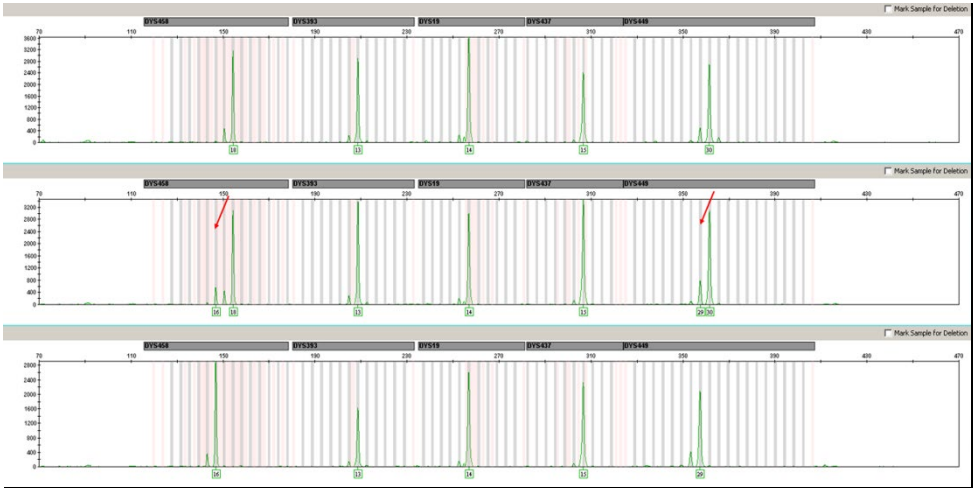
Locus	9948 genotype	QHID3 genotype
DYS389I	13	13
DYS391	10	11
DYS389II	31	30
DYS533	12	12
DYS390	24	23
DYS627	22	21
DYS458	18	16
DYS393	13	13
DYS19	14	14
DYS437	15	15
DYS449	30	29
DYS460	11	11
DYS576	16	16
YGATAH4	12	12
DYS481	24	22
DYS448	19	19
DYS518	38	38
DYS439	12	12
DYS549	13	12
DYS438	11	12
DYS456	17	15
DYS643	11	10
DYS570	18	18
DYS638	23	23
DYS385	11/14	11/14
DYS392	13	14

A



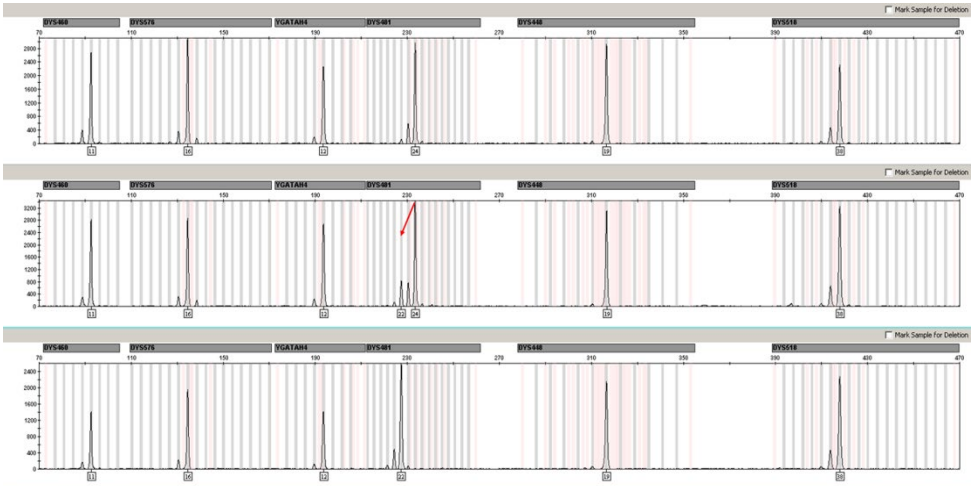
A. Expected bp positions of uncovered minor component peaks are indicated by red arrows. **DYS391**: Minor allele 11 assigned. **DYS389II**: Minor allele 30 overlaps with stutter position. **DYS390**: Minor allele 23 overlaps with stutter position. **DYS627**: Minor allele 21 overlaps with stutter position. DYS389I and DYS533 have the same allele.

B



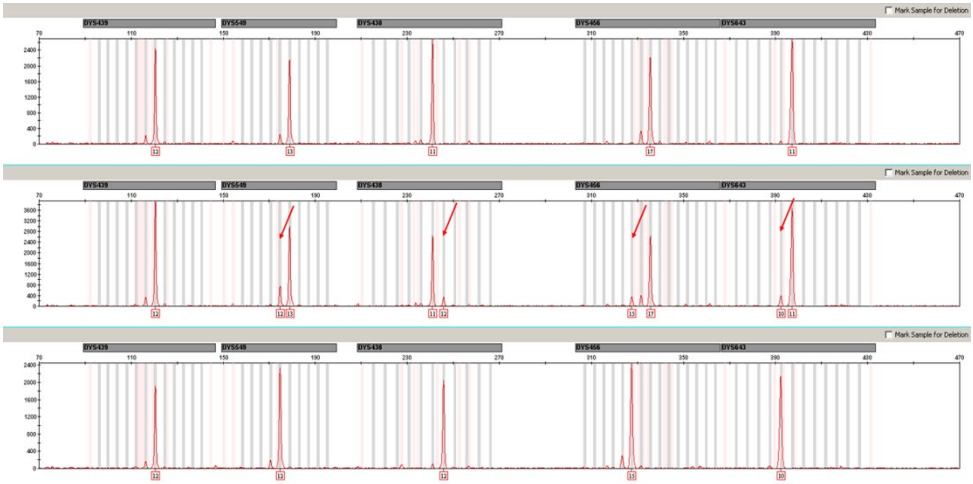
B. Expected bp positions of uncovered minor component peaks are indicated by red arrows. **DYS458**: Minor allele 16 can be assigned. **DYS449**: Minor allele 29 overlaps with stutter position. DYS393, DYS19, and DYS437 have the same allele.

C



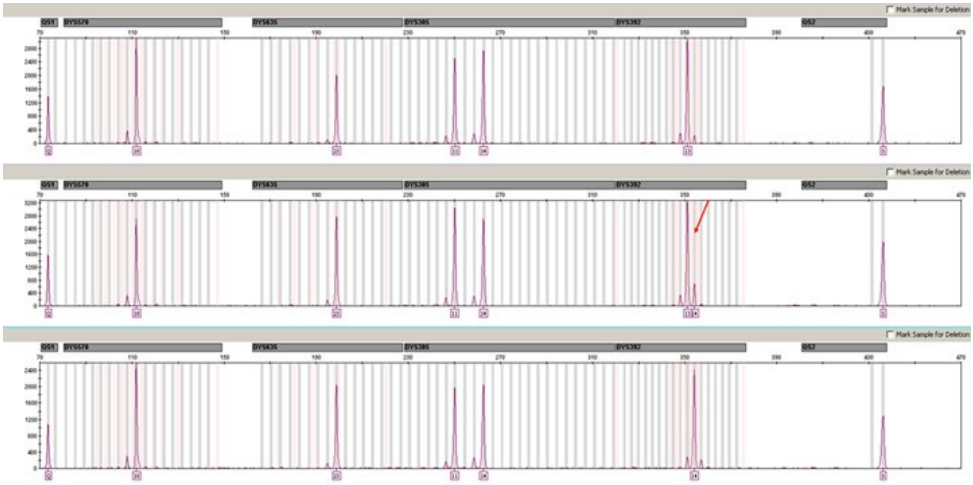
C. Expected bp positions of uncovered minor component peaks are indicated by red arrows. **DYS481**: Minor allele 22 can be assigned. DYS460, DYS576, YGATAH4, DYS448, and DYS518 have the same allele.

D



D. Expected bp positions of uncovered minor component peaks are indicated by red arrows. **DYS549**: Minor allele 12 overlaps with stutter position. **DYS438**: Minor allele 15 can be assigned. **DYS456**: Minor allele 12 can be assigned. **DYS643**: Minor allele 10 overlaps with stutter position. DYS439 has the same allele.

E



E. Expected bp positions of uncovered minor component peaks are indicated by red arrows. **DYS392**: Minor allele 14 overlaps with stutter position. DYS570, DYS635, and DYS385 have the same allele.

Figure 19. Results of the mixture analysis. Figures A–E show a 1:10 mixture of DNA QHID3 and control DNA 9948 (middle panel of each figure) and the corresponding single source samples as a reference (upper and lower panel of each figure). Red arrows indicate positions, where a peak of the minor component DNA that does not overlap with a main peak is to be expected. A brief description of the mixture situation given for each individual marker is given below the figures.

Robustness of direct amplification of reference samples

Following typical types of reference samples have been tested for compatibility:

- Blood on FTA (non-indicating QIAcard FTA)
- Buccal cells on FTA (EasiCollect)
- Buccal cells on Bode Buccal Collector
- Buccal cells on swabs (Sarstedt)

Samples were taken from 10 different donors each, and amplified in duplicates. The reactions took place under standard conditions specified in the Investigator Argus Y-28 QS Kit Handbook Appendix. All samples gave full profiles. Typical profile examples are shown (Figures 20–23).

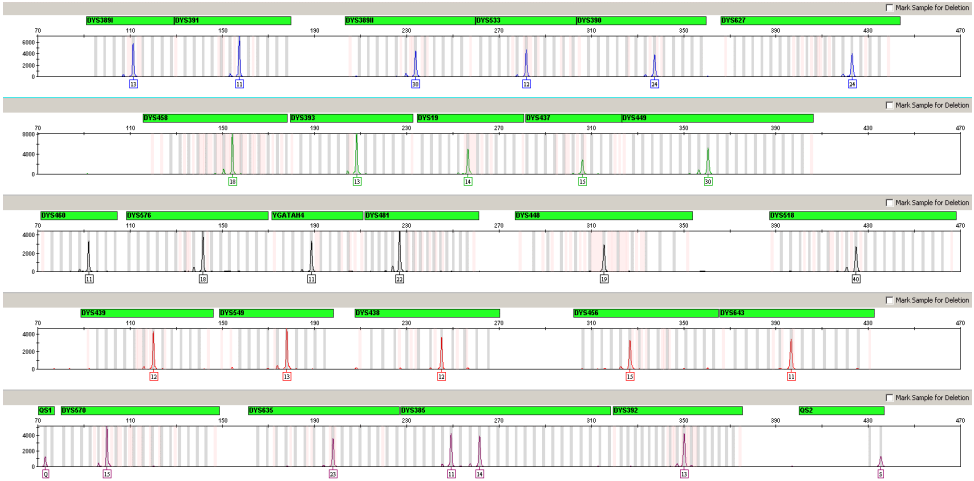


Figure 20. Typical example of a Buccal cells on swabs sample.

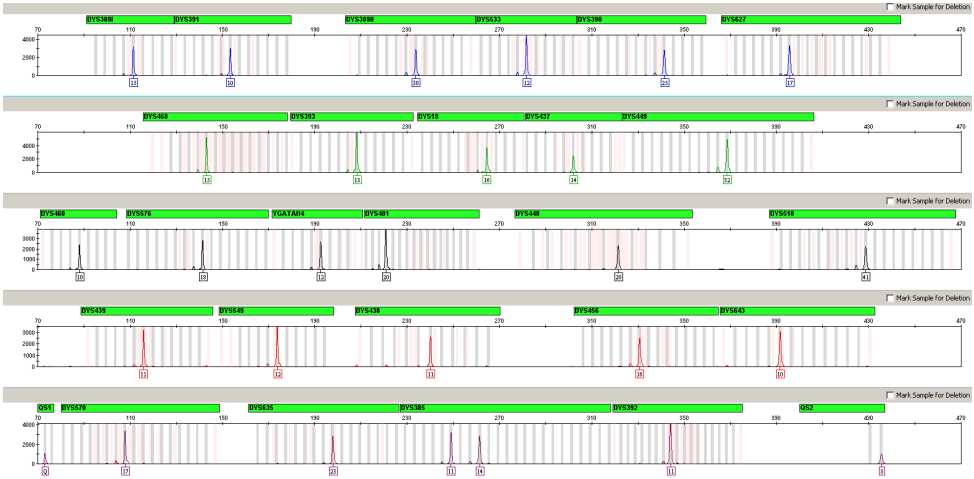


Figure 21. Typical example of a Blood on FTA sample.

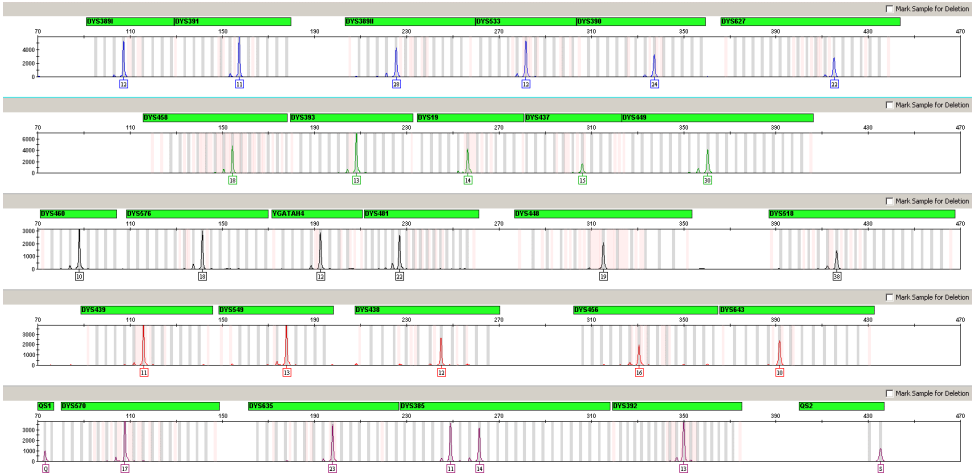


Figure 22. Typical example of an EasiCollect sample.

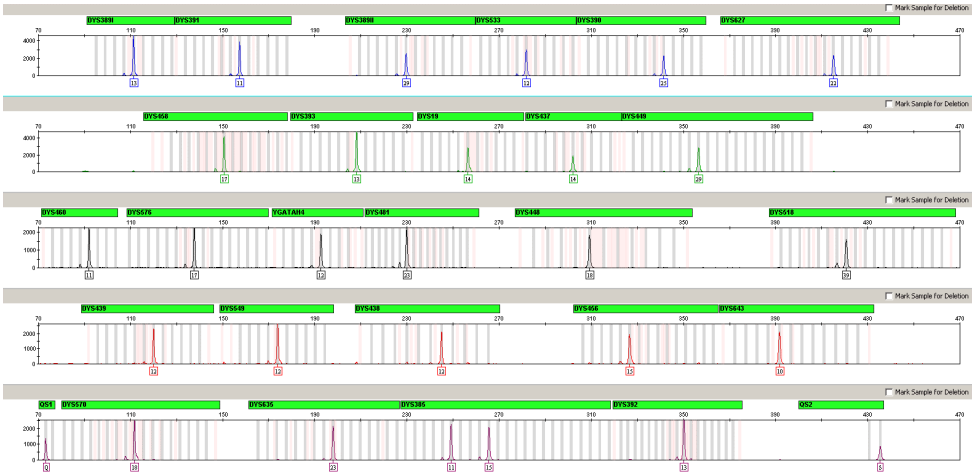
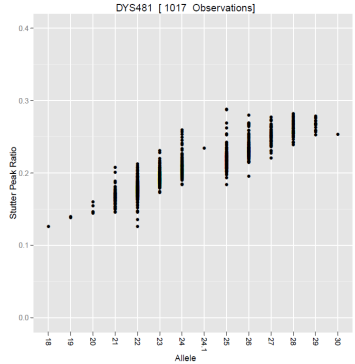


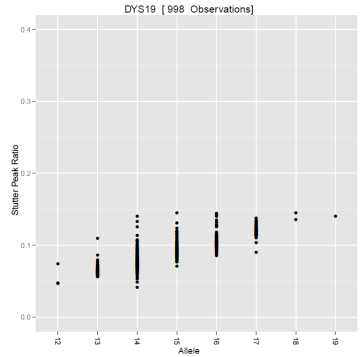
Figure 23. Typical example of a Bode Buccal Collector sample.

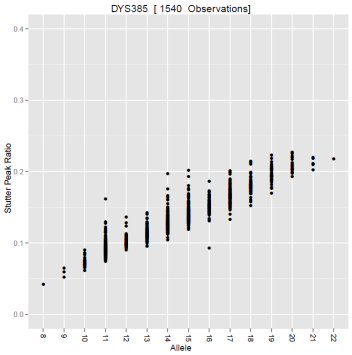
Reproducibility: Concordance test

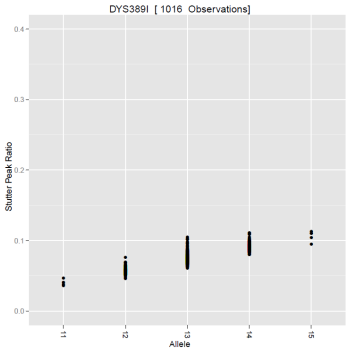
A concordance study was conducted at the National Institute of Standards and Technology (NIST). A set of 1062 samples from unrelated male individuals was used. Profiles obtained with the Investigator Argus Y-28 QS Kit were compared to profiles previously obtained with the Yfiler Plus Kit (Thermo Fisher Scientific), the PowerPlex® Y23 Kit (Promega Corporation), and Verogen's ForenSeq DNA Signature Prep Kit. Out of 28,674 alleles compared, three discordant calls were observed, resulting in a concordance rate of 99.9999%. Two of the disconcordant alleles were due to null alleles (DYS460, DYS456). For DYS481, one sample was typed as 24.1, where the other kits typed allele 25.

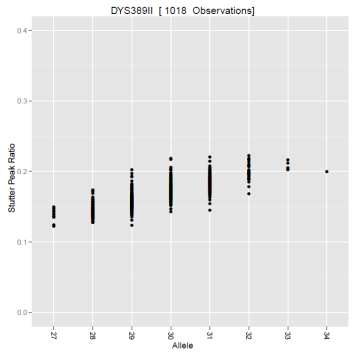
The concordance study results were also used to calculate stutter peak heights (Figure 24). Please note that the marker DYS643 produces stutters at a barely noticeable level. No stutter event exceeding the analysis threshold was observed in this study, even when lowering the threshold to 30 RFU. The stutter filter values provided in the template files were deduced from a smaller internal study with samples intentionally pushed to higher profile peak heights.

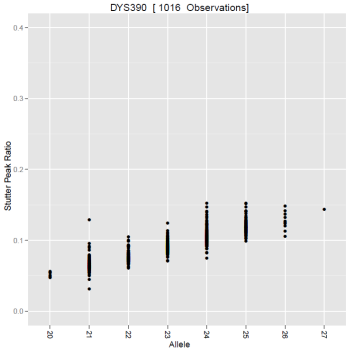

DYS481 [1017 Observations]
Stutter Peak Ratio
Allele

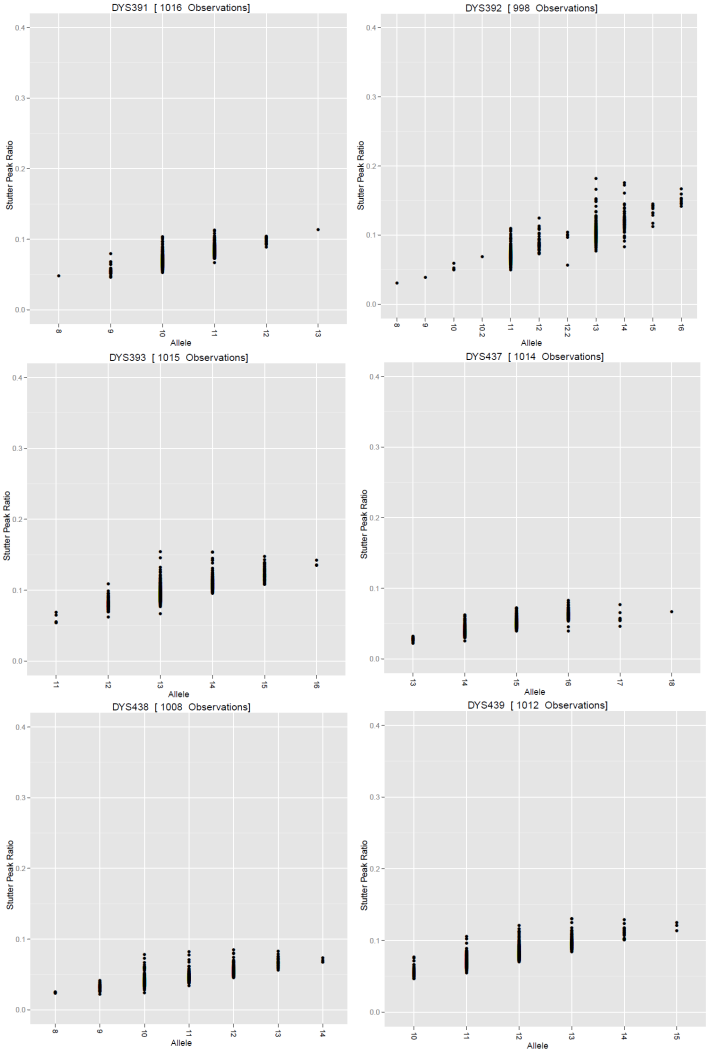

DYS19 [998 Observations]
Stutter Peak Ratio
Allele

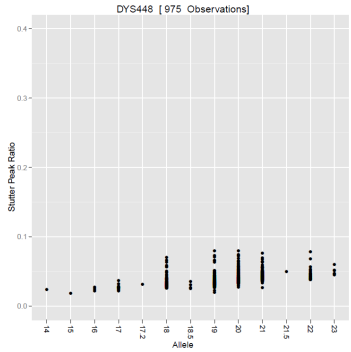

DYS385 [1540 Observations]
Stutter Peak Ratio
Allele

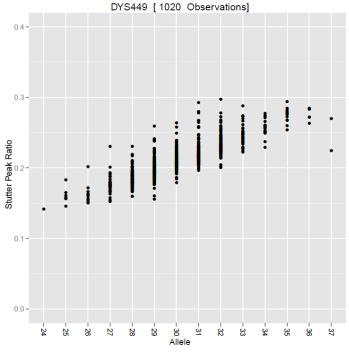

DYS389I [1016 Observations]
Stutter Peak Ratio
Allele

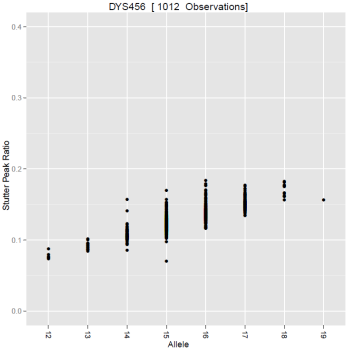

DYS389II [1018 Observations]
Stutter Peak Ratio
Allele

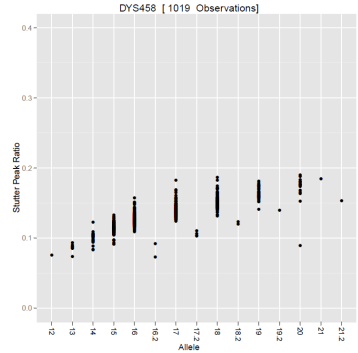

DYS390 [1016 Observations]
Stutter Peak Ratio
Allele

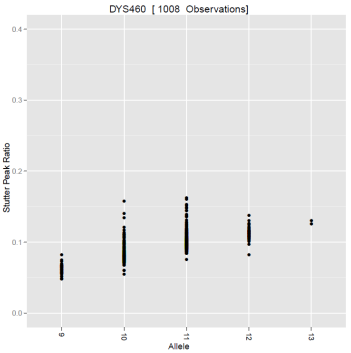

DYS391 [1016 Observations]
DYS392 [998 Observations]
DYS393 [1015 Observations]
DYS437 [1014 Observations]
DYS438 [1008 Observations]
DYS439 [1012 Observations]
Stutter Peak Ratio
Allele

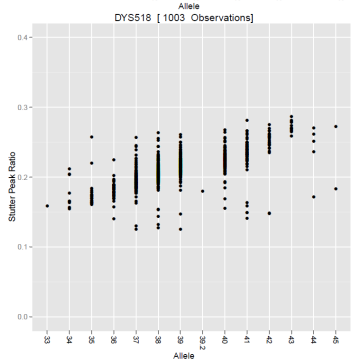

DYS448 [975 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
14
15
16
17
17.2
18
18.3
19
20
21
21.3
22
23
Allele

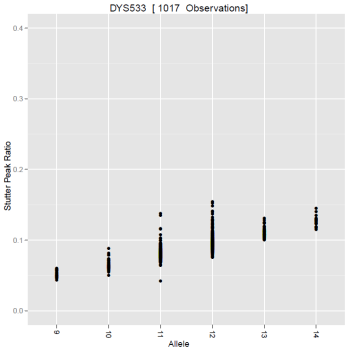

DYS449 [1020 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
24
25
26
27
28
29
30
31
32
33
34
35
36
37
Allele

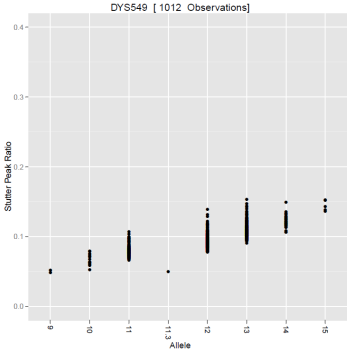

DYS456 [1012 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
12
13
14
15
16
17
18
19
Allele

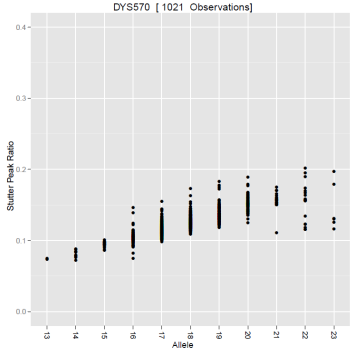

DYS458 [1019 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
12
13
14
15
16
16.2
17
17.2
18
18.2
19
19.2
20
21
21.2
Allele

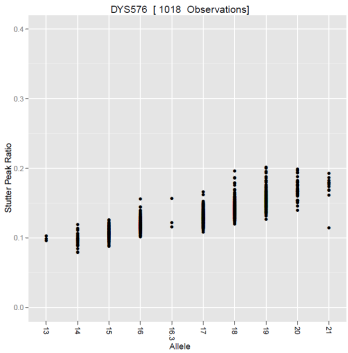

DYS460 [1008 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
9
10
11
12
13
Allele


DYS518 [1003 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
33
34
35
36
37
38
39
39.2
40
41
42
43
44
45
Allele


DYS533 [1017 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
9
10
11
12
13
14
Allele


DYS549 [1012 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
9
10
11
11.3
12
13
14
15
Allele


DYS570 [1021 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
13
14
15
16
17
18
19
20
21
22
23
Allele


DYS576 [1018 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
13
14
15
16
16.3
17
18
19
20
21
Allele

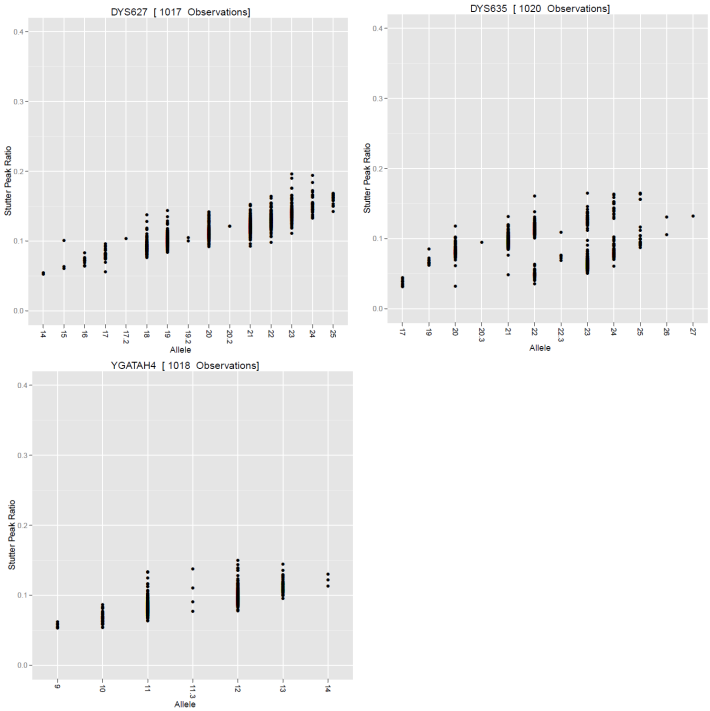


Figure 24. Stutter peak height ratios. Peak heights of forward stutters compared to main peaks were analyzed for 1062 DNA samples from different donors.

References

1. ENFSI Forensic Guidelines. (2010). Recommended Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process. Ref. Code: ENFSI DNA WORKING GROUP. https://enfsi.eu/wp-content/uploads/2016/09/minimum_validation_guidelines_in_dna_profiling_-_v2010_0.pdf.
2. Revised Validation Guidelines of Scientific Working Group on DNA Analysis Methods (SWGDAM), 2016.
3. Kayser, M. et al. (1997). Evaluation of Y-chromosomal STRs: a multicenter study. Int. J. Legal. Med. **110**, 125.
4. Gusmão, L., et al. (1999). Y chromosome specific polymorphisms in forensic analysis. Leg. Med. (Tokyo). **1**, 55.
5. Beleza, S., et al. (2003). Extending STR markers in Y chromosome haplotypes. Int. J. Legal. Med. **117**, 27.
6. Redd, A., et al. (2002). Forensic value of 14 novel STRs on the human Y chromosome. Forensic. Sci. Int. **130**, 97.
7. Balantyne, K., et al. (2012). A new future of forensic Y-chromosome analysis: rapidly mutating Y-STRs for differentiating male relatives and paternal lineages. Forensic. Sci. Int. Genet. **6**, 208.

Ordering Information

Product	Contents	Cat. no.
Investigator Argus Y-28 QS Kit (100)	Primer mix, fast reaction mix 3.0, control DNA, allelic ladder Argus Y-28, DNA Size Standard, and nuclease-free water	383625
Investigator Argus Y-28 QS Kit (400)	Primer mix, fast reaction mix 3.0, control DNA, allelic ladder Argus Y-28, DNA Size Standard, and nuclease-free water	383627

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Document Revision History

Date	Changes
12/2021	Initial revision

HB-2994-001 12/2021