QIAGEN Validation Report

Developmental validation of the Investigator® IDplex Plus Kit

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

The performance of the Investigator IDplex Plus Kit was evaluated with regard to various sample types and conditions commonly encountered in forensic and paternity testing 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 15 polymorphic STR markers: the 13 Combined DNA Index System (CODIS) markers (TH01, D3S1358, vWA, D21S11, TPOX, D7S820, D5S818, D16S539, CSF1PO, D13S317, FGA, D18S51, D8S1179), plus D2S1338, D19S433, and the gender-specific Amelogenin. These genetic loci have been characterized in numerous studies by other laboratories (see reference 3 for review). Optimal reaction conditions were established, and the effects of variations in those conditions were assessed. The kit was tested in house and at independent external forensic laboratories.

The effects of variations in buffer component concentrations are described using the example of the $MgCl_2$ concentration (page 2). The robustness of the assay regarding variations in PCR cycling conditions and on a range of different thermal cyclers and genetic analyzers was investigated (pages 4–10). 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 11).

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 or degraded DNA (pages 13 and 18). Cross-reactivity with non-human DNA (page 19) was also assessed. The reproducibility of the results was verified (page 26).

Results of developmental validation

The validation study was performed at a QIAGEN R&D facility. All of the electropherograms shown were generated on an Applied Biosystems® 3500™ Genetic Analyzer. The standard conditions specified in the *Investigator IDplex Plus Handbook* were used for the electrophoresis. Unless stated otherwise, a GeneAmp® PCR System 9700 with Gold-plated Silver 96-Well Block was used for amplification. Data were analyzed using the QIAGEN Investigator IDproof Software, v2.0 or Applied Biosystems GeneMapper® *ID-X* software, v1.2.

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 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 XY1 (Figure 1). The assay yielded robust results within an $MgCl_2$ concentration range of ±30% of the optimum.

Under these conditions, amplification of the markers was well balanced and no dropout or nonspecific 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).

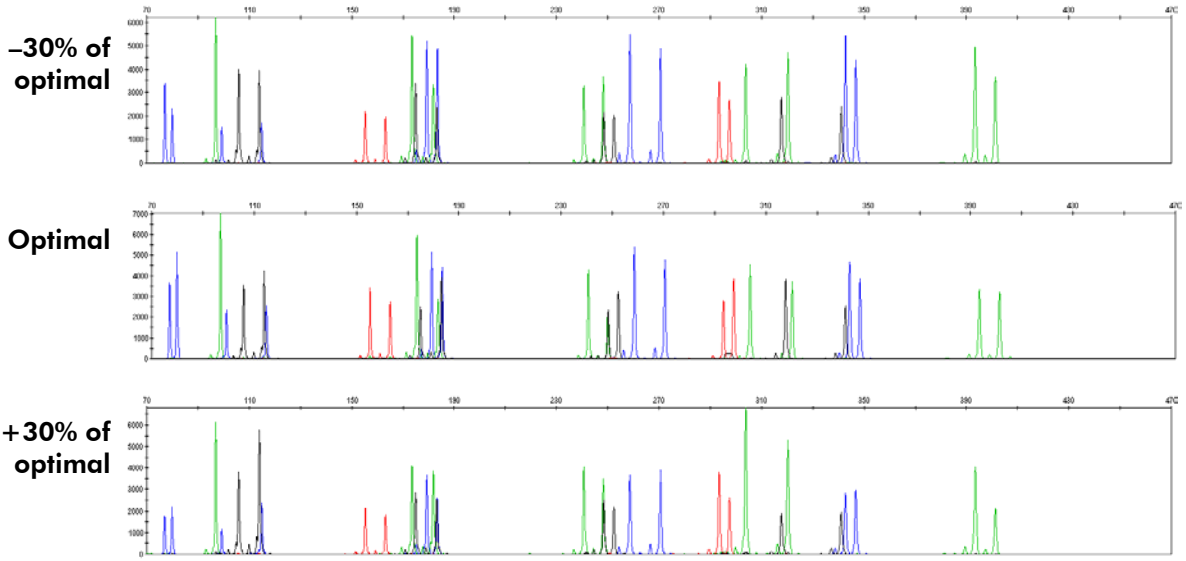


Figure 1. Influence of $MgCl_2$ concentration. Examples of sample duplicates are shown.

Effect of PCR annealing temperature variations

Specificity, sensitivity, and robustness are critical in forensic casework, and all are affected by the annealing temperature (T_m). 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 IDplex Plus reaction (61°C).

Annealing temperatures between 55°C and 67°C were applied to the amplification of 500 pg Control DNA XY1 and PCR was performed on an Eppendorf® Mastercycler® ep instrument. Reactions using annealing temperatures between 55°C and 64.5°C resulted in full profiles. Good inter-locus balance was observed for the temperature range 58.5–61.5°C. However, the average peak height of markers was best for the conditions closest to the actual annealing temperature of 61°C (Figure 2 and Figure 3). The first dropouts were observed at 66°C. No nonspecific PCR products were observed above an annealing temperature of 57°C. In order to ensure optimal performance of the assay, we strongly recommend regular calibration of thermal cyclers.

Similar sets of experiments were performed to set optimal temperatures and hold times for all PCR protocol steps (data not shown).

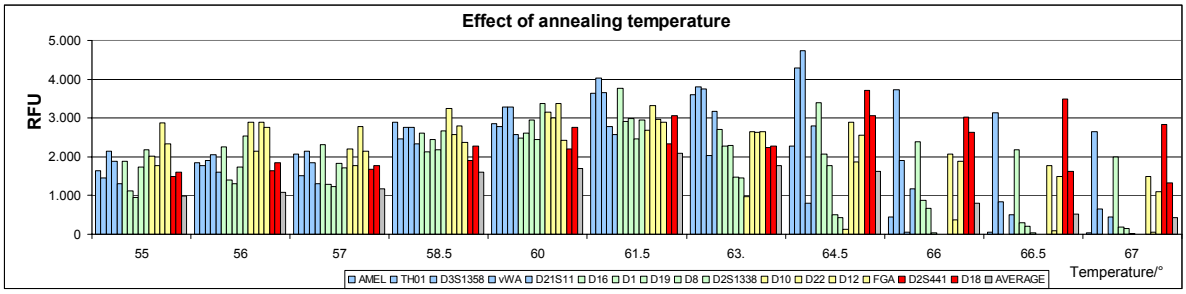


Figure 2. Effect of variations in the PCR annealing temperature. Each bar represents an average peak height of duplicates. Bar colors represent the individual markers, gray bars show the average of all markers.

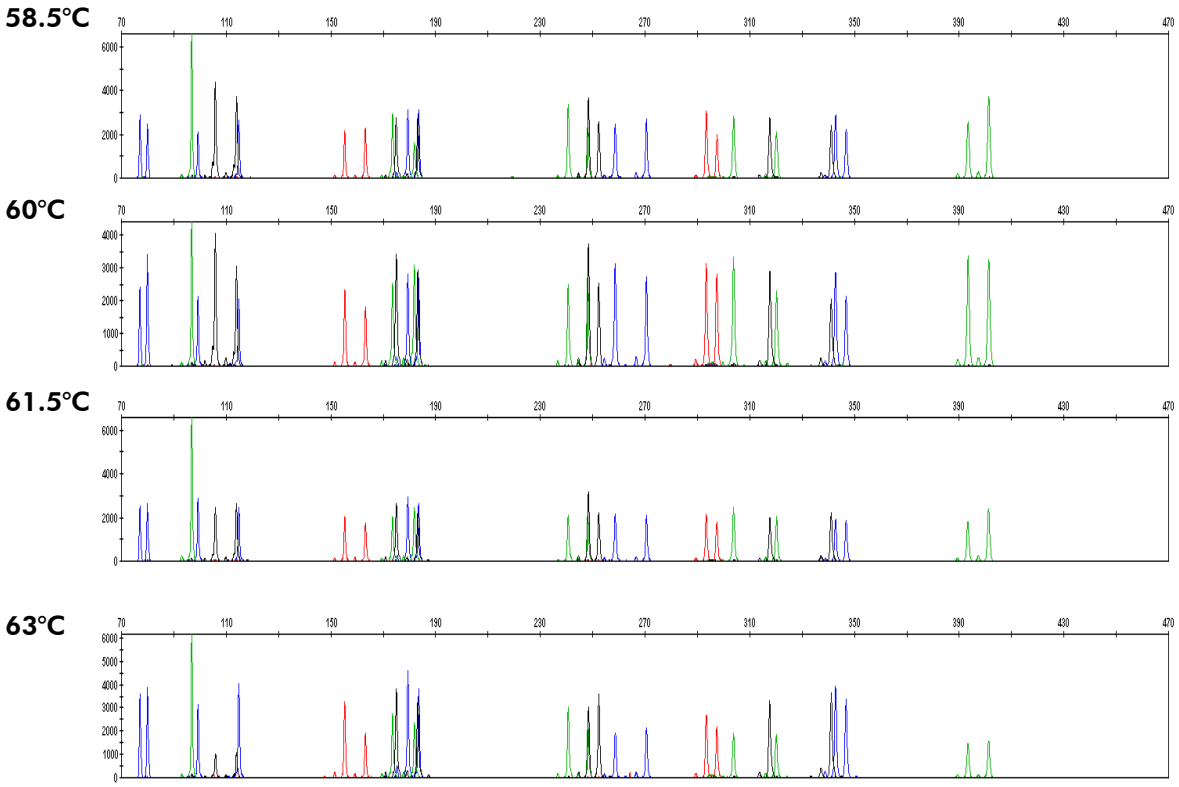


Figure 3. Variations in the PCR annealing temperature. Representative electropherograms for reactions using annealing temperatures of 58.5–63°C are shown. **Note**: The x-axis was scaled for best fit.

Effect of different cycle numbers

Altering the number of PCR cycles can be used 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 speed up the protocol when there is abundant DNA in the sample (e.g., for database samples).

Cycle numbers were increased to 32 (or 34 for reactions containing 32, 16, or 8 pg Control DNA XY1), and the numbers of called alleles and the peak heights were compared to those from a standard 30-cycle protocol (Figure 4). As expected, the signal intensities of the amplified products increase with higher cycle numbers. However, it should be noted that an increase in cycle number to more than 30 will not necessarily result in more information obtained from the low-template-DNA sample. Furthermore, because of stochastic effects, increased peak imbalances or dropouts may in general be observed for low-copy-number samples (with 100 pg or less template DNA), regardless of any increase in cycle numbers. In this experiment, using a threshold of 50 RFU for allele calling, the number of allelic dropouts due to stochastic effects was not significantly reduced when more PCR cycles were applied (Figure 4). Note that the sensitivity of the capillary electrophoresis instrument and the setting of the threshold of detection strongly influence the outcome of an assessment of the use of increased cycle numbers. Therefore, such protocol adaptations must be evaluated by individual laboratories using their instruments. Adapting the injection time of the capillary electrophoresis instrument or using post-PCR purification (e.g., the QIAGEN MinElute® PCR Purification Kit) are alternative methods for increasing sensitivity.

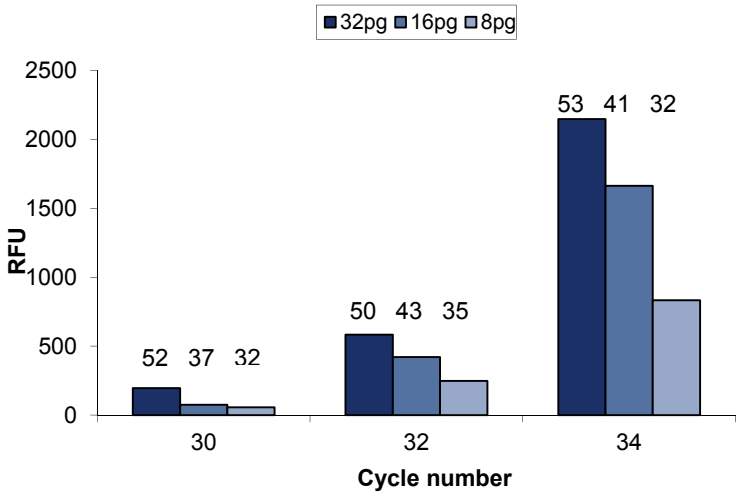


Figure 4. Effect of different cycle numbers on mean peak height and number of called alleles. DNA XY1 was used as the template in the different amounts indicated. Samples were run in duplicate and the numbers of detected PCR products (indicated above the bars; 62 PCR products expected) and their peak heights calculated. 50 RFU was used as a threshold for detection.

In particular, for reference samples such as buccal swabs, where DNA can be extracted in abundance, reduced cycle numbers may be used to streamline the laboratory workflow. Here, cycle numbers were decreased to 24, 26, or 28 for reactions containing 2.5 ng or 10 ng template DNA (Figure 5). All reactions resulted in robust amplification and full profiles were obtained using a threshold of 50 RFU for allele calling. However, 28 cycle amplifications of 10 ng DNA gave rise to pull-up peaks when applying samples to analysis without prior dilution of the PCR product. An example electropherogram for a 24-cycle amplification of 10 ng template DNA is shown in Figure 6, next page.

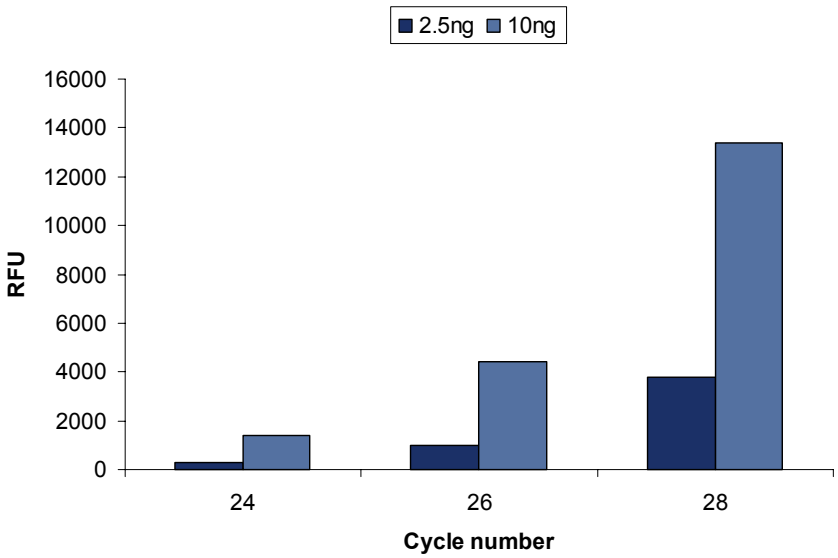


Figure 5. Effect of decreased cycle numbers on mean peak heights. 2.5 ng or 10 ng Control DNA XY1 were subjected to amplification using 24, 26, or 28 PCR cycles. Samples were run in duplicate and average peak heights calculated.

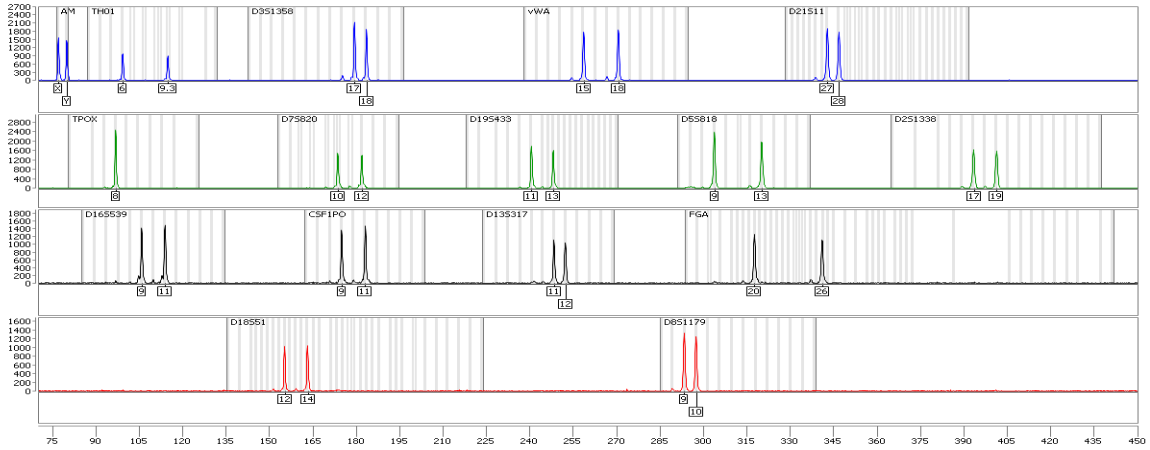


Figure 6. Reduction of PCR cycle numbers with elevated template DNA amounts. Results for amplification of 10 ng Control DNA XY1 using 24 instead of the standard 30 PCR cycles. Data were analyzed using QIAGEN Investigator IDproof Software.

Effect of different cycler types

Several PCR thermal cyclers were tested with the Investigator IDplex Plus Kit to demonstrate kit robustness independent of the instrument (Figure 7, next page). 2 ng to 32 pg Control DNA XY1 was used as a PCR template. The reaction took place under standard conditions (30 cycles) and was performed with the following thermal cyclers.

- GeneAmp PCR System 9700 with Aluminum 96-Well Block (Applied Biosystems Inc., Foster City, CA, USA)
- GeneAmp PCR System 9700 with Silver or Gold-plated Silver 96-Well Block (Applied Biosystems Inc., Foster City, CA, USA)
- MJ Research DNA Engine® PTC-200 Peltier Thermal Cycler (Bio-Rad Laboratories GmbH, Munich, Germany)
- UNO-Thermo Block (Biometra biomedizinische Analytik GmbH, Göttingen, Germany)
- QIAGEN Rotor-Gene® Q

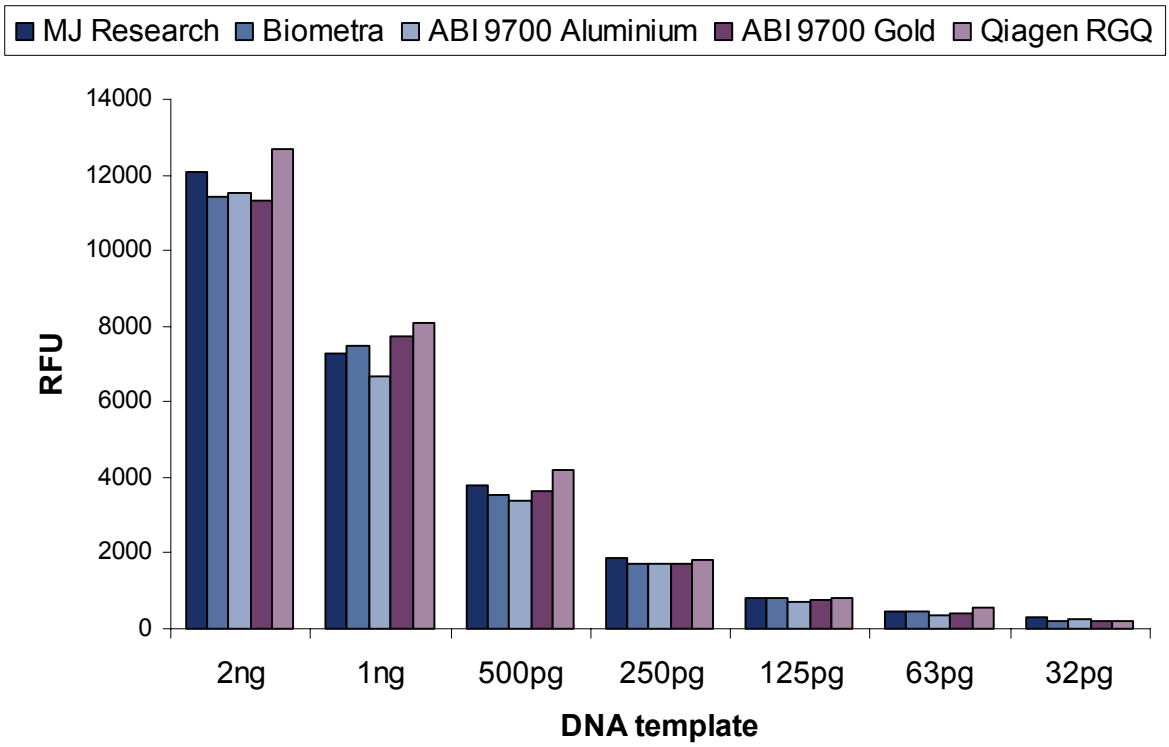


Figure 7. Performance on different PCR thermal cyclers. DNA XY1 was used as the template in the different amounts indicated. Each sample was run in duplicate. A standard 30-cycle protocol was used. Average peak heights across all markers are shown.

Comparable mean peak heights were obtained for all of the tested PCR cyclers. No significant differences in intra- and inter-locus balance or nonspecific amplification were observed on any of the thermal cyclers. In the case of the GeneAmp PCR System 9700, it is essential to use the "Standard mode" in order to achieve optimal results. Do not use the "9600 emulation mode".

Effect of different genetic analyzers

Several genetic analyzers were tested with the Investigator IDplex Plus Kit in order to demonstrate its robustness. 500 pg Control DNA XY13 and Control DNA 9948 were each amplified in duplicate. The reaction took place under standard conditions and the PCR products were tested on all genetic analyzers in parallel:

- Applied Biosystems 3500 Genetic Analyzer (2 independent instruments)
- Applied Biosystems 3130 Genetic Analyzer
- ABI PRISM 310 Genetic Analyzer

The electropherograms in Figure 8 show comparable mean peak heights for all of the tested genetic analyzers. No imbalance, dropouts, or preferential amplification for the STR systems was observed on either genetic analyzer.

Similar overall peak heights and a comparable balance of peaks between individual markers of the same color channel, as well as between different channels, were observed (Figure 8). Variations reflected differences in sensitivity between individual instruments, e.g., due to laser power, array life, and matrix calibration effects. Limitations of genetic analyzer sensitivity must addressed by individual laboratories during an internal validation study using the instrumentation in their laboratory.

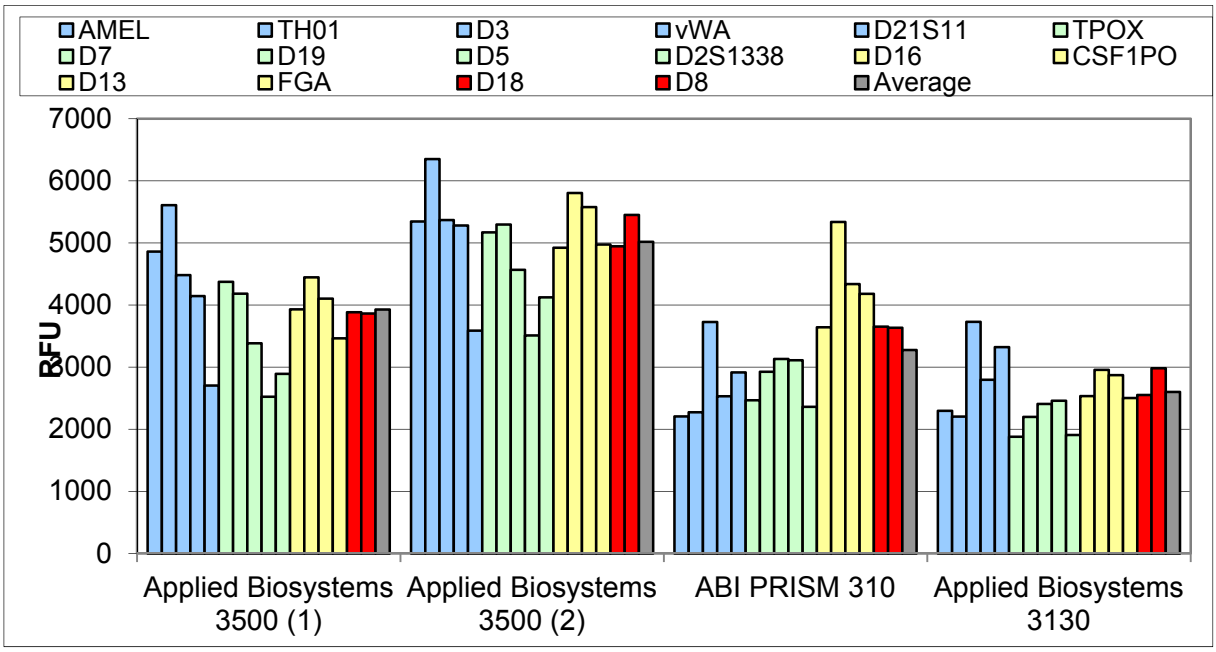


Figure 8. Analysis of PCR products on different genetic analyzers. 500 pg Control DNA 9948 and XY13 was amplified on a GeneAmp PCR System 9700 in duplicate. Samples were run according to the *Investigator IDplex Plus Handbook*. Average peak heights for duplicates of both DNAs are shown.

Sensitivity

The Investigator IDplex Plus 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, e.g., using the QIAGEN Investigator Quantiplex Kit. In particular, for heavily degraded DNA, the use of increased template amounts may improve results (see page 18).

Control DNA XY1 was serially diluted from 2 ng to 8 pg per reaction. Full profiles (31 PCR products) were consistently obtained at 125 pg using the standard conditions specified in the *Investigator IDplex Plus Handbook*. Occasional allele dropouts were found due to stochastic effects when ≤63 pg DNA was used as the template. As expected, the number of dropouts increases with decreasing DNA concentration. See "Effect of different cycle numbers", page 6, 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 >1 ng do not cause issues during PCR amplification, those samples still can result in inaccurate or unusable data. Depending on the instrumentation and settings used for capillary electrophoresis, fluorescence intensity may go off the scale.

"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 gets 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 reamplifying 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, e.g., caused by dye artifacts. Figure 9 (next page) shows an example 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.

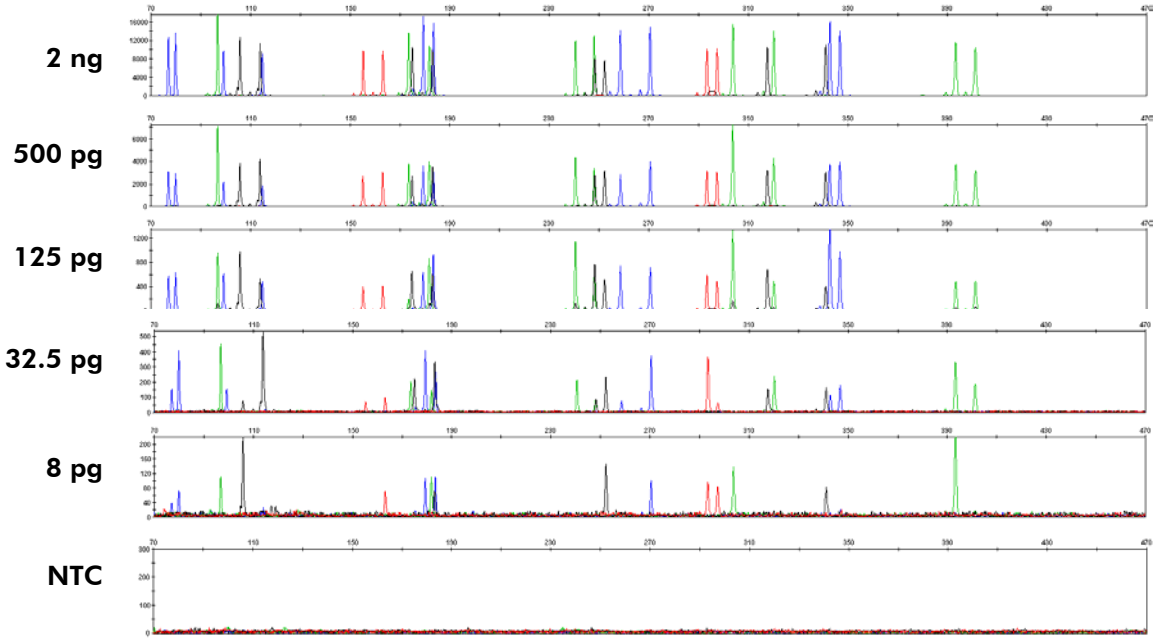


Figure 9. Sensitivity study. Serial dilutions of Control DNA XY1 were analyzed. The amounts of DNA indicated were used as the template for amplification. **Note**: Y-axis scales were adjusted for best fit. The lowest panel shows a no-template (negative) control to illustrate the expected level of background fluorescence.

Performance with simulated inhibition

If the DNA extraction from forensic casework samples is done using inappropriate methods, Investigator IDplex Plus assay 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.

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 with 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

Results are shown in Figures 10–13. Figure 14 shows an overview of Investigator IDplex Plus inhibitor resistance.

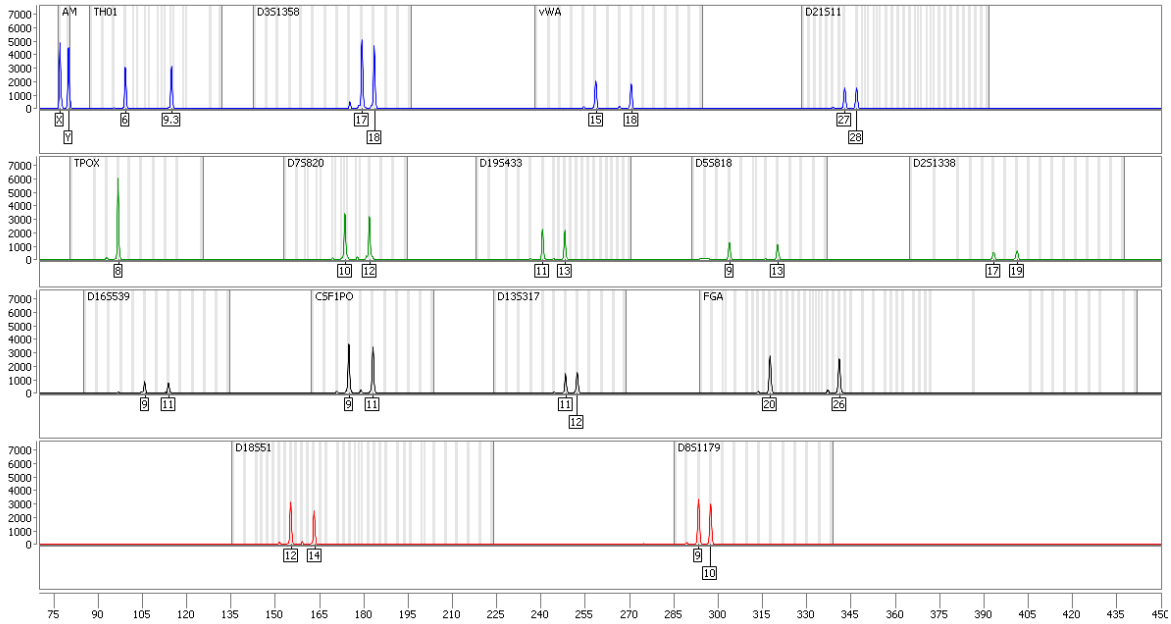


Figure 10. Electropherogram of 500 pg Control DNA XY1 amplified in the presence of 175 ng/µl humic acid. Data were analyzed using QIAGEN Investigator IDproof Software.

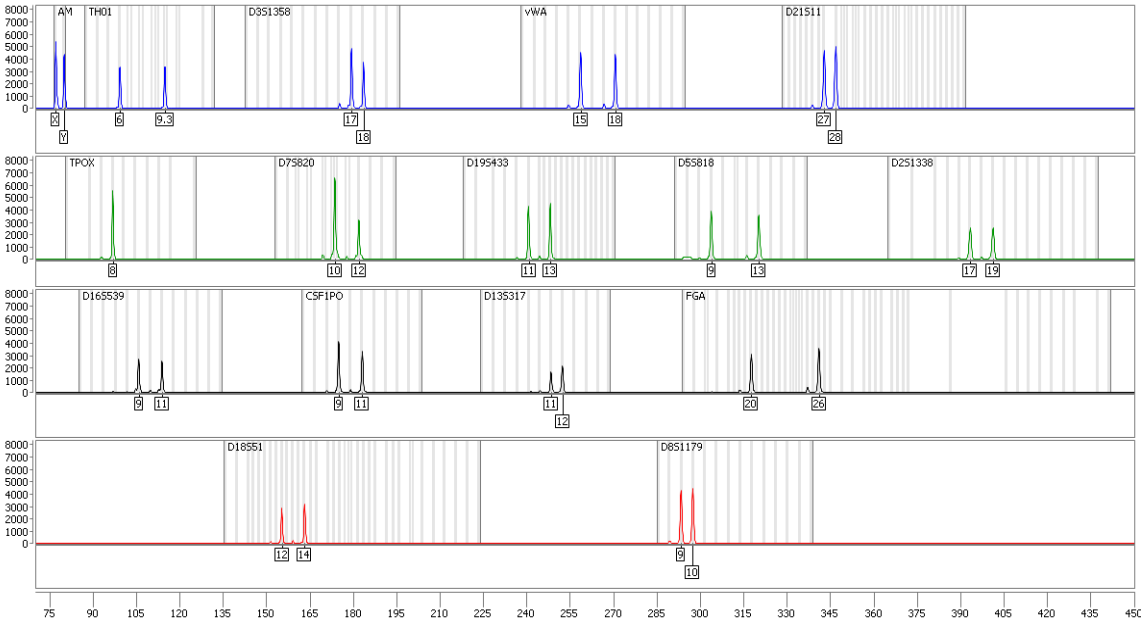


Figure 11. Electropherogram of 500 pg Control DNA XY1 amplified in the presence of 500 µM hematin. Data were analyzed using QIAGEN Investigator IDproof Software.

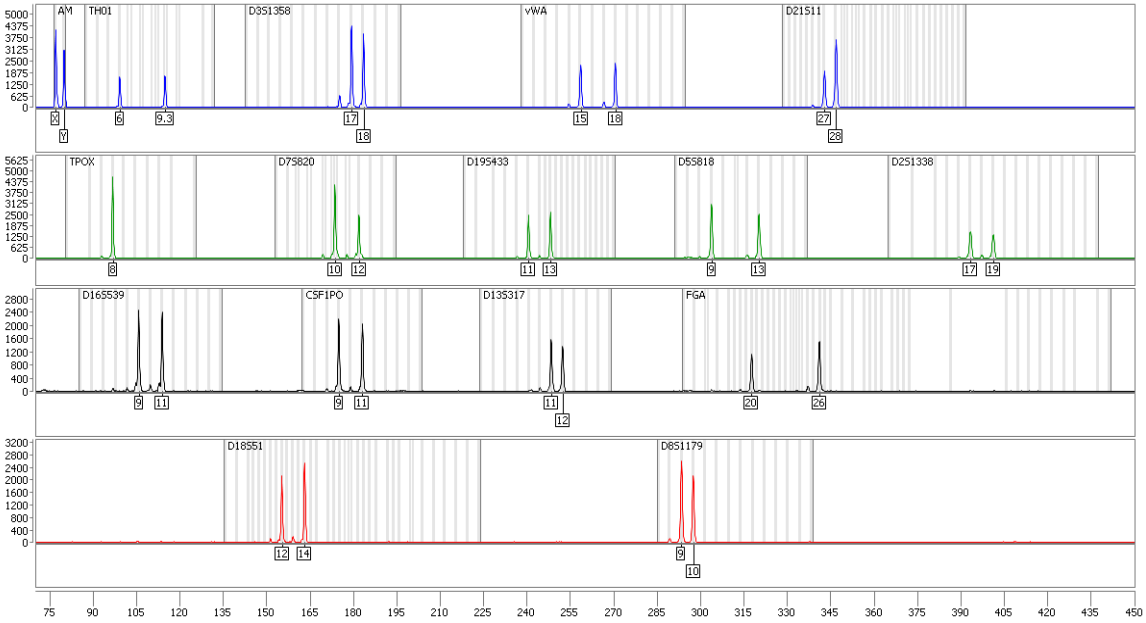


Figure 12. Electropherogram of 500 pg Control DNA XY1 amplified in the presence of 8 mM indigo carmine. Data were analyzed using QIAGEN Investigator IDproof Software.

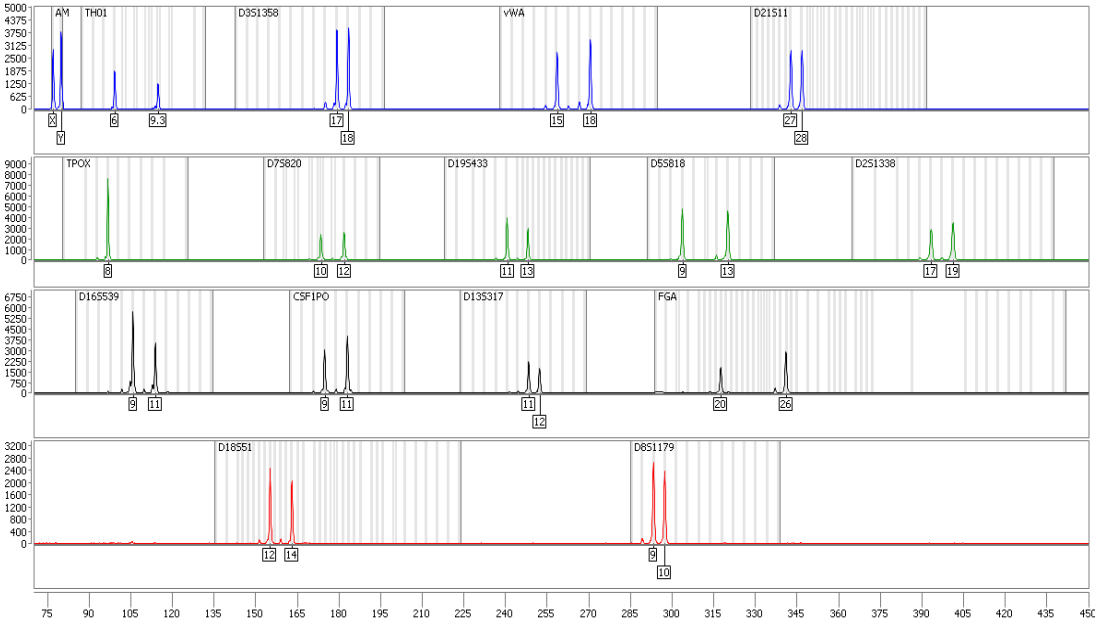


Figure 13. Electropherogram of 500 pg Control DNA XY1 amplified in the presence of 150 ng/µl collagen. Data were analyzed using QIAGEN Investigator IDproof Software.

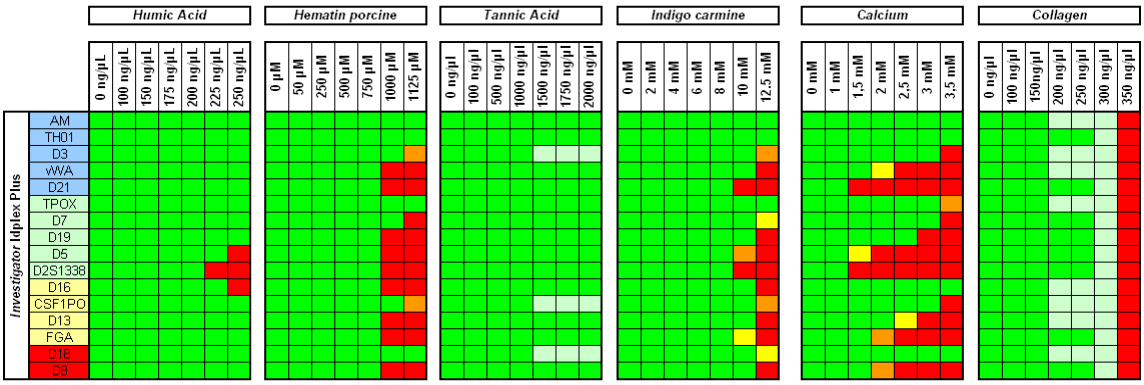


Figure 14. Overview of Investigator IDplex Plus inhibitor resistance. The assay was tested for its robustness toward inhibitors (humic acid, hematin, tannic acid, indigo carmine, calcium, and collagen). 500 pg Control DNA XY1 was used as the template and PCR was performed under standard conditions. 100 RFU was used as threshold for allele calling. **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. **Light green**: Consistently full profile with split peaks.

Full profiles lacking any PCR artifacts, e.g., split peaks, were obtained over a wide range of inhibitor concentrations. For most inhibitors (e.g., humic acid and hematin), 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. Tannic acid shows a different mode of interference with PCR amplification. It affects the ability of the polymerase to adenylate the final PCR product. As a consequence, the presence of –A products leads to split peaks that are visible in electropherograms, while the overall amount of amplification product is not significantly reduced (Figure 15, next page). This effect is sequence dependent, so markers are affected differently.

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.

500 ng/µl

1500 ng/µl

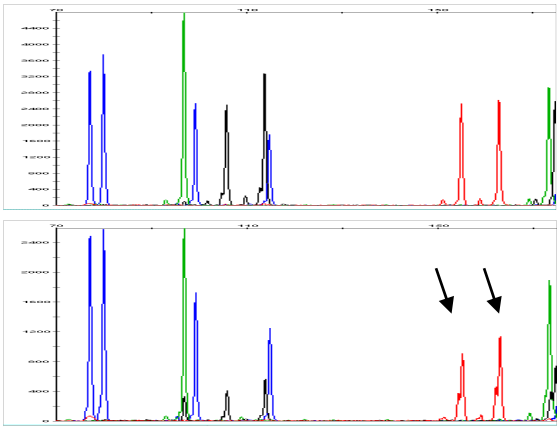


Figure 15. Effect of high levels of tannic acid. 500 pg Control DNA XY1 was amplified in the presence of 500 or 1500 ng/µl tannic acid. Arrows indicate PCR products showing split peaks caused by partial adenylation.

Stability with degraded DNA

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

Artificially degraded male genomic DNA was used for the 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. Fragment lengths were verified on an agarose gel and 500 pg DNA was used as the template for amplification (all samples in duplicate) (Figure 16A). Full profiles were obtained with DNA with an average length of 300 bp and 500 bp. As expected, loci with larger amplicon sizes become affected as DNA degradation progresses. At an average fragment length of 150 bp, 45 out of 62 expected peaks were detected using a threshold of 50 RFU (Figure 16B). Increased amounts of template (up to 2 ng) can be used to improve results for heavily degraded DNA.

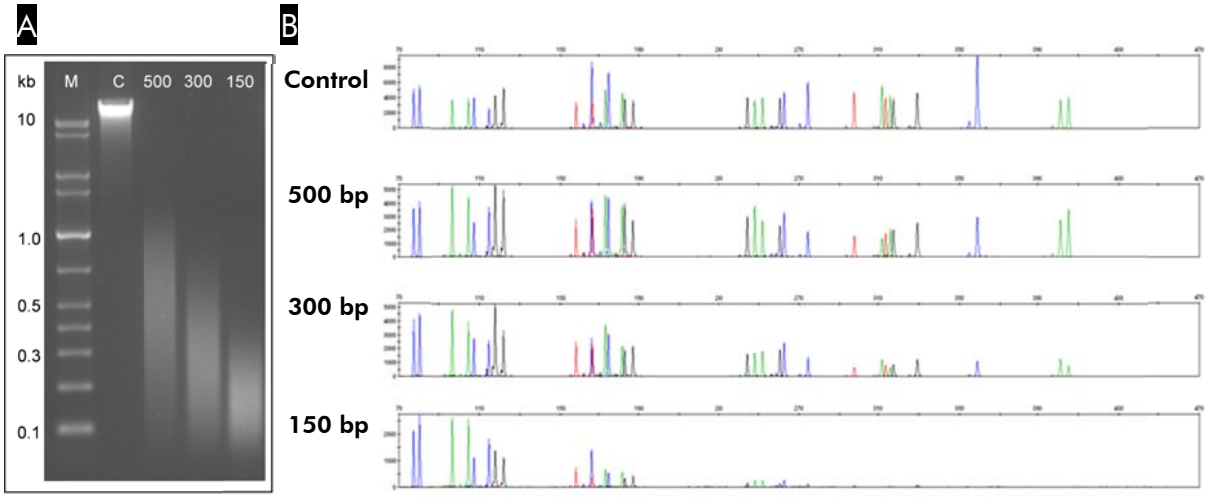


Figure 16. Degraded DNA. Male DNA was sheared to the defined average fragment length indicated. **A** Agarose gel analysis of degraded DNA. **M**: DNA size standard markers, **C**: Untreated DNA. 500, 300, 150: average fragment lengths in base pairs. **B** STR analysis of untreated control and degraded DNA samples. 500 pg template DNA was used. Full profiles were obtained down to an average fragment length of 300 bp.

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 IDplex Plus species specificity for human DNA, DNA from other species was each tested following the standard assay protocol (Figure 17, next page).

Besides common pets and farm animals, some primates were also tested. As expected for primates, amplification of some products is possible. Chimpanzee, bonobo, orangutan, and to a lesser degree, gorilla give rise to several peaks within marker ranges in all channels, some of which match the size of human STR products. Macaque DNA produced an Amelogenin X-peak and further off-ladder peaks in the FAM™, BTG, and BTR panel.

Horse showed a low-level off-ladder peak in the BTG panel at 105 bp. Cow, goat, and sheep showed a low-level off-ladder peak in the BTG panel at 124 bp. An additional off-ladder peak was shown for sheep in the FAM panel at 271 bp.

Pig DNA produced a single peak of about 300 bp detected in the FAM panel, located between vWA and D21S11.

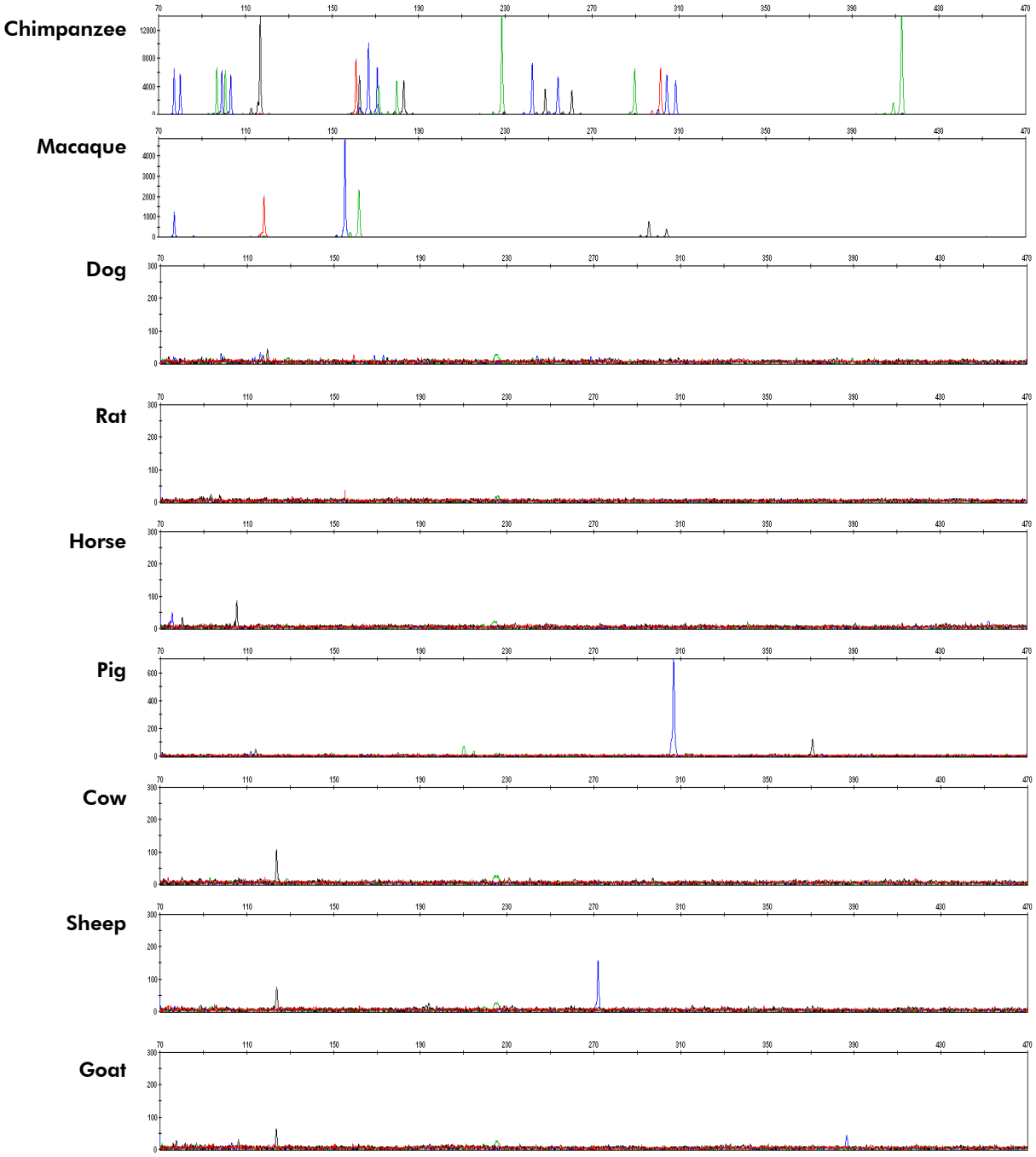


Figure 17. Representative results of the species specificity assessment. 500 pg primate DNA and 2.5 ng DNA from all other species was used as the template.

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 control DNA XY5 and XY13 in ratios of 1:1, 3:1, 7:1, 10:1, 15:1, and vice versa (see Table 1, next page, for the genotypes of the mixed samples). The total amount of mixed DNA used in this study was 500 pg; a 15:1 mixture thus contains 31 pg of the minor component and 469 pg of the major component (Table 2, next page). The limit of detection of the minor component was determined by analyzing non-overlapping alleles of both types of DNA. 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 1:10 mixtures is shown in Figure 18 (pages 23–25). 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 (Figure 19, page 25) if the amount of available DNA is not limited. See "Sensitivity", page 11, for general considerations.

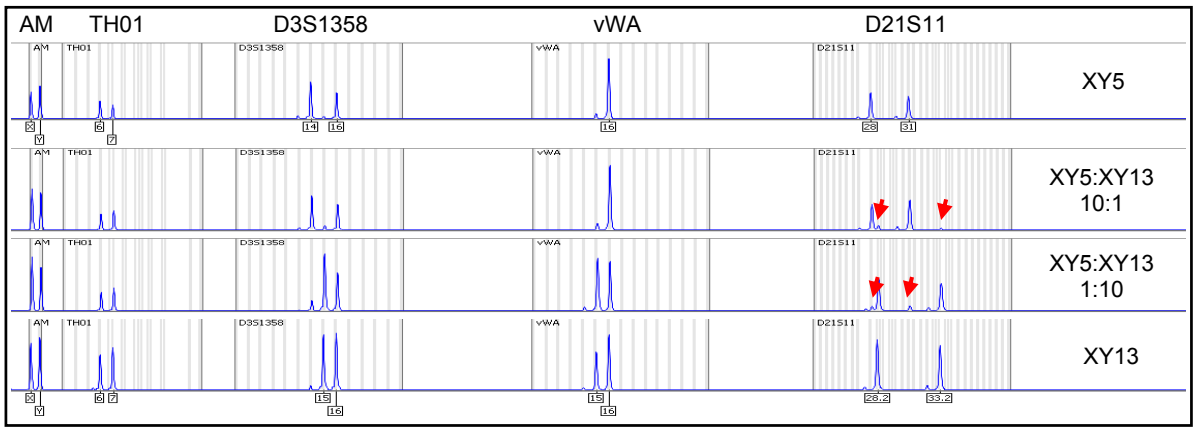
Table 1. Genotypes of DNA used for mixed samples

Locus	XY5 genotype	XY13 genotype
Amelogenin	X/Y	X/Y
D2S1338	16/19	17/23
D3S1358	14/16	15/16
D5S818	11/12	11/11
D7S820	10/10	10/12
D8S1179	12/15	13/14
D13S317	11/12	12/14
D16S539	9/12	11/12
D18S51	13/15	14/15
D19S433	13/14	13/14
D21S11	28/31	28.2/33.2
CSF1PO	11/12	10/10
FGA	20/25	22/23
TH01	6/7	6/7
TPOX	8/11	8/8
vWA	16/16	15/16

Table 2. Amounts of DNA template in mixtures

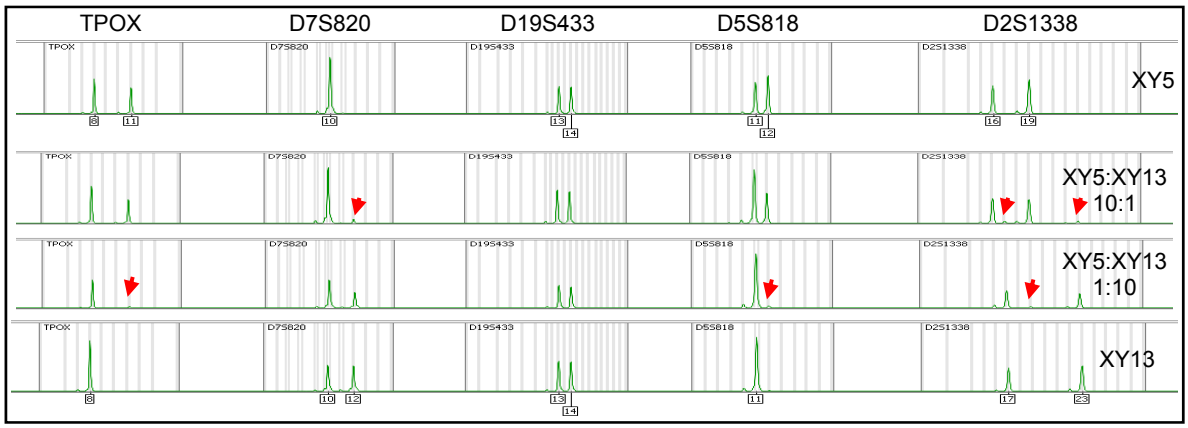
Mixture ratio	Major component	Minor component
1:1	250 pg	250 pg
3:1	375 pg	125 pg
7:1	437 pg	63 pg
10:1	450 pg	50 pg
15:1	469 pg	31 pg

A



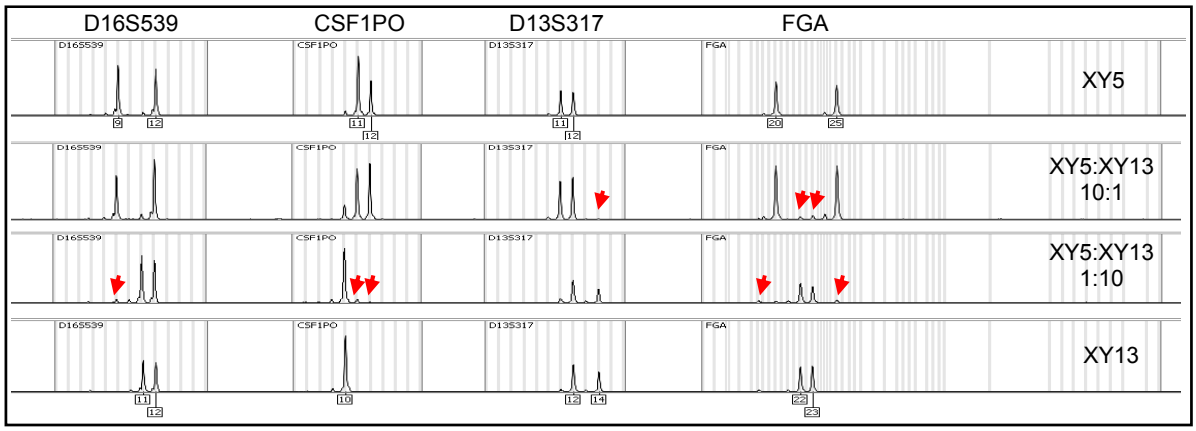
Amelogenin, TH01: Heterozygote + heterozygote, 4 overlapping alleles. Major component completely covers minor component. D3S1358: Heterozygote + heterozygote, 2 overlapping alleles, 2 alleles overlapping with stutter positions. Minor component is covered by major component, or visible as elevated stutter. vWA: Homozygote + heterozygote, 2 overlapping alleles, 1 allele overlapping with stutter position. Minor component is covered by major component, or visible as elevated stutter. D21S11: Heterozygote + heterozygote, no overlapping alleles. All minor component peaks can be assigned (arrows).

B



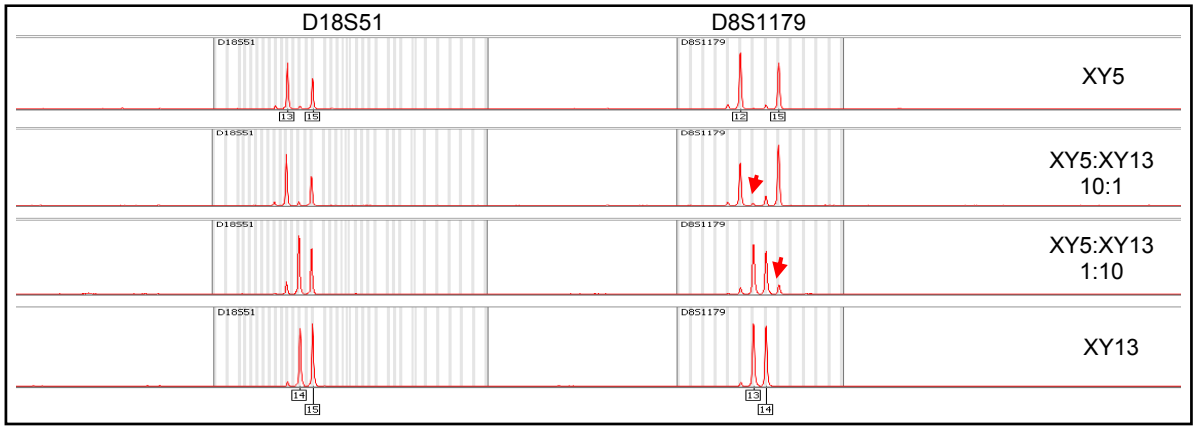
TPOX: homozygote + heterozygote, 2 overlapping alleles. The non-overlapping minor component peak can be assigned (arrow). D7S820: homozygote + heterozygote, 2 overlapping alleles. The non-overlapping minor component peak can be assigned (arrow). D19S433: heterozygote + heterozygote, 4 overlapping alleles. Major component completely covers minor component. D5S818: homozygote + heterozygote, 2 overlapping alleles. The non-overlapping minor component peak can be assigned (arrow). D2S1338: heterozygote + heterozygote, no overlapping alleles, 1 allele overlapping with stutter position. 3 non-overlapping minor component peaks can be assigned (arrow).

C



D16S539: heterozygote + heterozygote, 2 overlapping alleles, one allele overlapping with stutter position. The non-overlapping minor component peak can be assigned (arrow). CSF1PO: homozygote + heterozygote, no overlapping alleles. One allele overlapping with stutter position, 2 non-overlapping minor component peaks can be assigned (arrows). D13S317: Heterozygote + heterozygote, two overlapping alleles, 1 allele overlapping with stutter position. One non-overlapping minor component peak can be assigned, but peak height is very close to 50 RFU threshold (arrow). See also Figure. 20. FGA: Heterozygote + heterozygote, no overlapping alleles. All 4 non-overlapping minor component peaks can be assigned (arrows).

D



D18S51: Heterozygote + heterozygote, 2 overlapping alleles, 2 alleles overlapping with stutter positions. Minor component is covered by major component, or visible as elevated stutter. D8S1179: heterozygote + heterozygote, no overlapping alleles, 2 alleles overlapping with stutter positions. The 2 non-overlapping minor component peaks can be assigned (arrows).

Figure 18. Results of the mixture analysis. A–D show 1:10 and 10:1 mixtures of DNA XY5 and XY13 (middle panels 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, or a forward stutter peak of the major component, is to be expected. A brief description of the mixture situation given for each individual marker is given below the figures. All but one of the expected peaks were found with a threshold of detection of 50 RFU. In this example, only allele 14 of D13S317 of DNA XY13 dropped out (Figure 18C, second panel).

Figure 19 shows an example of how such dropouts can be recovered by using more template DNA.

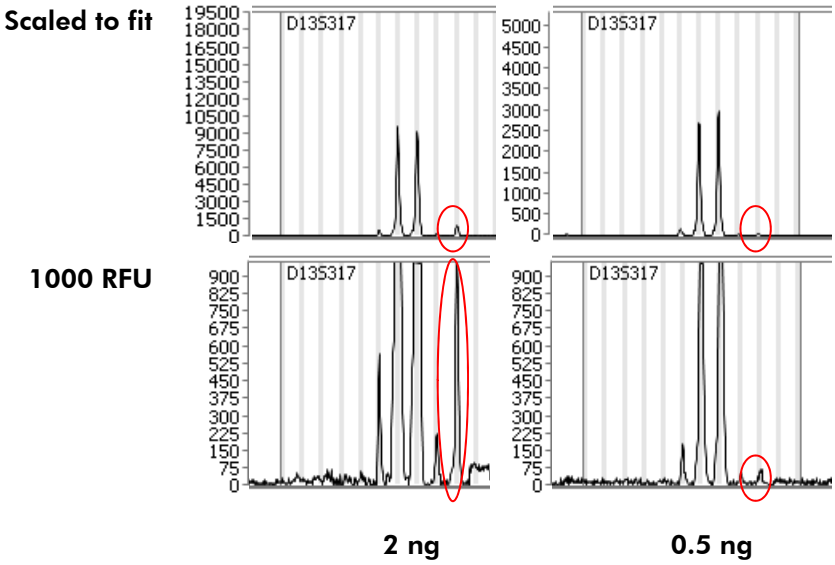


Figure 19. Analysis of DNA mixtures: Effect of increased template amount. Control DNA XY13 and XY5 were mixed in a 1:10 ratio. 0.5 ng or 2 ng of the mixture was amplified. Marker D2S1338 is shown; the main peaks correspond to allele 16 and 19 of DNA XY5. Allele 17 and 23 of the minor component DNA XY13 are indicated with red circles. Signal ratios of minor and major components are unchanged (top panel), while the signal of the minor component is pushed up against background when increased template is used (lower panel).

Reproducibility — concordance test

All primer binding sites of the Investigator IDplex Plus Kit are the same as in the Investigator IDplex assay. Minor changes (5' tailing of unlabeled primers) have been introduced to some sequences in order to optimize adenylation of PCR products and allow faster PCR cycling.

A concordance study with an internal DNA pool of 608 Caucasian samples in total was conducted. Identical DNA profiles were obtained for all samples using either the Investigator IDplex or IDplex Plus Kit, indicating that the minor primer changes between the two primer sets do not affect allele calls. The internal DNA pool was previously analyzed using the Applied Biosystems AmpFℓSTR® Identifiler™ PCR Kit. Genotypes obtained for all loci and Amelogenin were fully concordant for all but 3 samples. These non-concordant samples were previously typed as homozygous allele 13, but revealed a heterozygous 12/13 genotype when analyzed with the IDplex Plus Kit. No nonspecific amplification was observed in any of the samples.

Peak height ratios of heterozygous markers (Figure 20) and stutter peak heights (Figure 21, pages 27–29) were analyzed for a subset of 105 samples of the internal DNA pool.

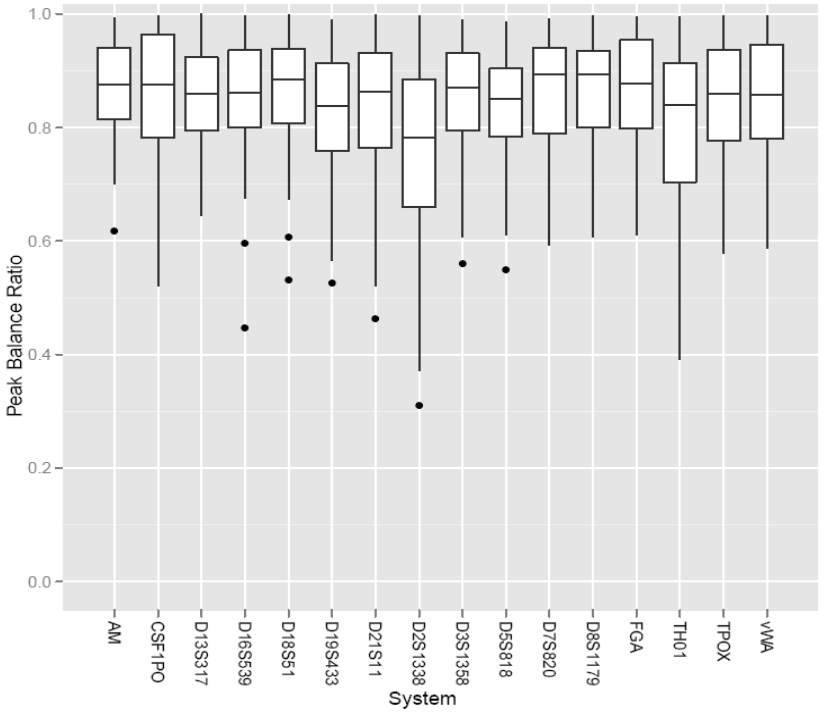
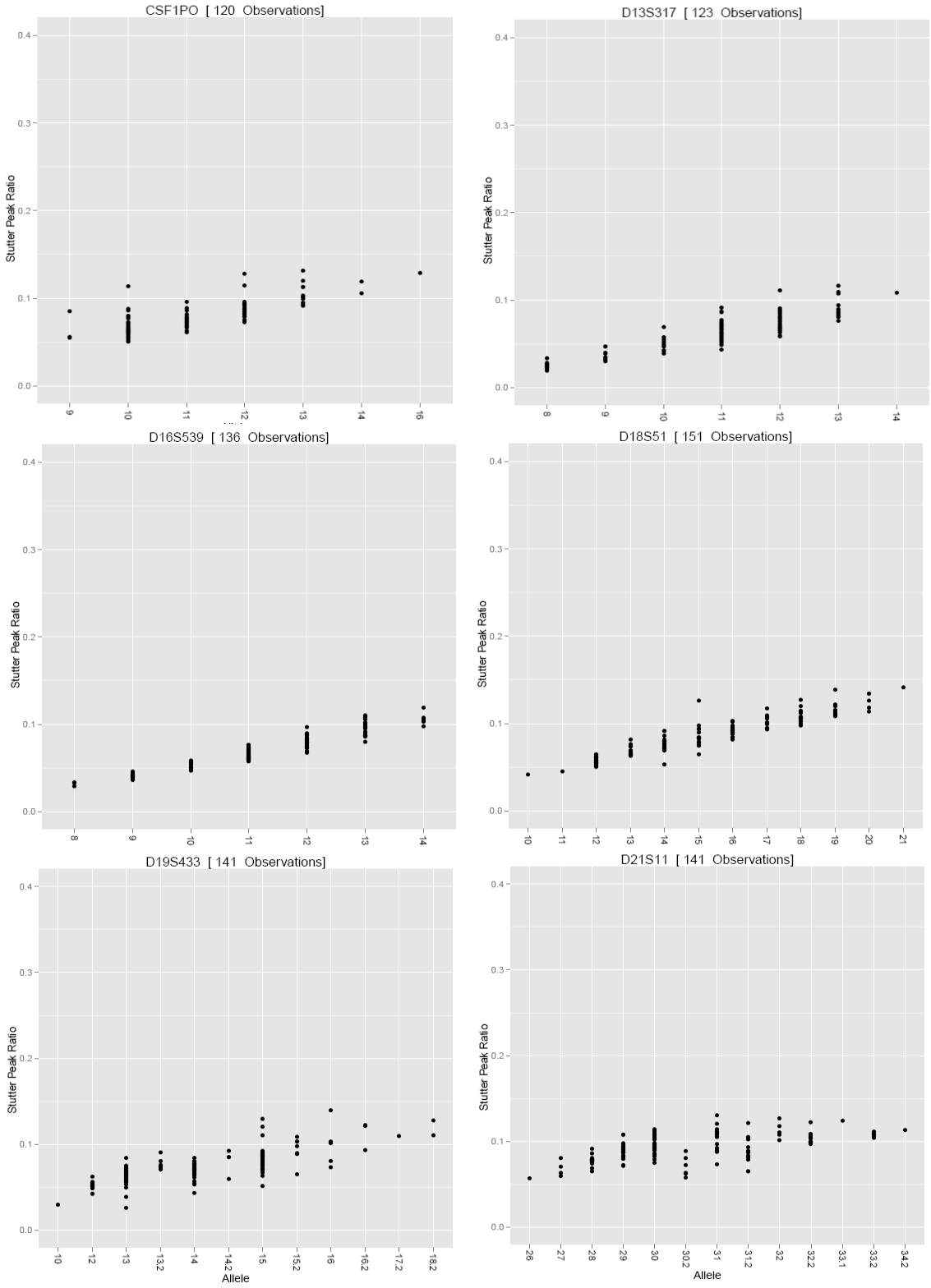
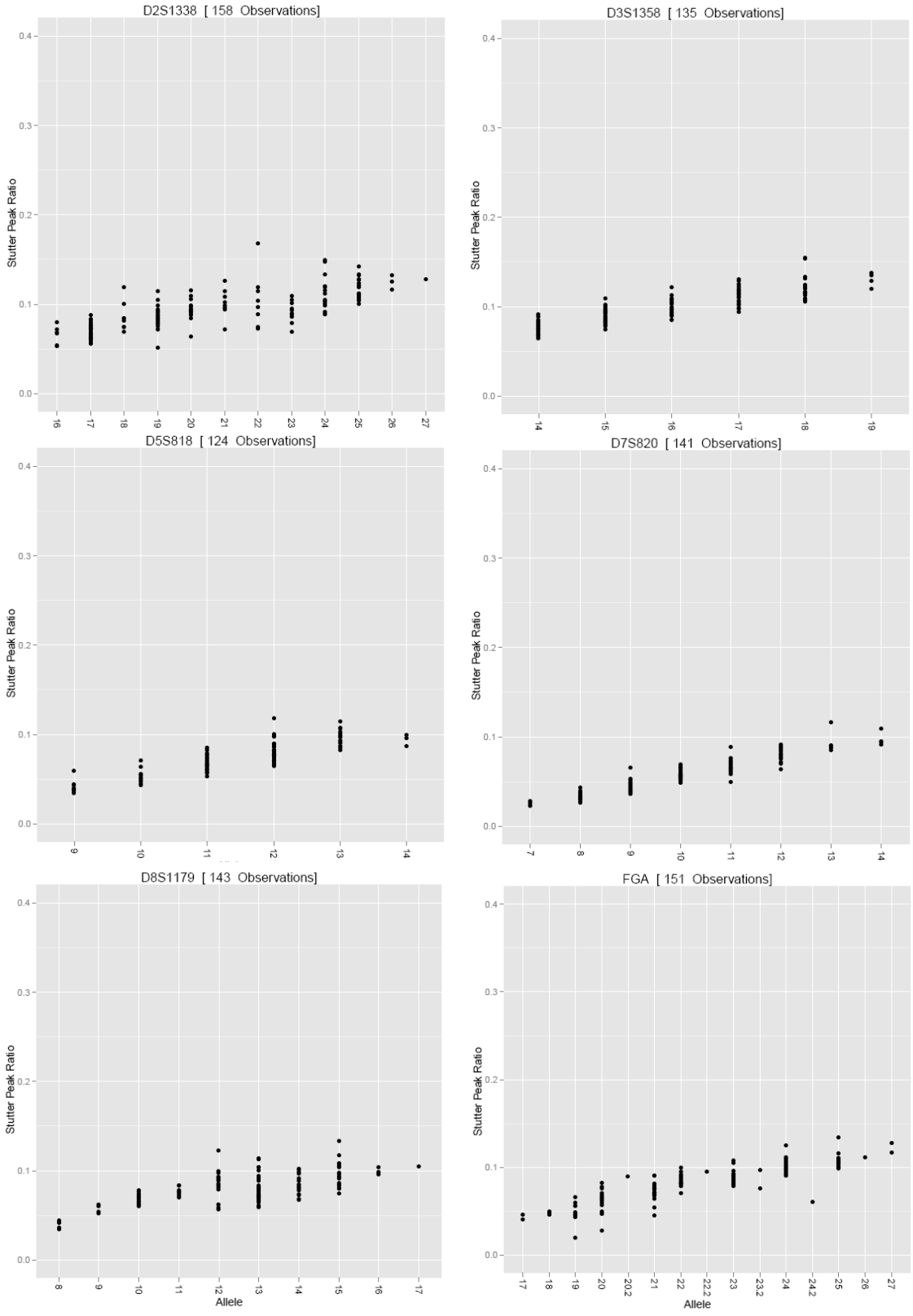


Figure 20. Box plot analysis of intra-locus peak height ratios. Values refer to the lower of the 2 peaks in a heterozygous sample. Boxes represent the middle 50% (interquartile range [IQR]) of data, and the lines inside the boxes represent the median. Whiskers show data within 1.5 IQR, dots represent outliers.


CSF1PO [120 Observations]
D13S317 [123 Observations]
D16S539 [136 Observations]
D18S51 [151 Observations]
D19S433 [141 Observations]
D21S11 [141 Observations]
Stutter Peak Ratio
Allele


D2S1338 [158 Observations]
D3S1358 [135 Observations]
D5S818 [124 Observations]
D7S820 [141 Observations]
D8S1179 [143 Observations]
FGA [151 Observations]
Stutter Peak Ratio
Allele

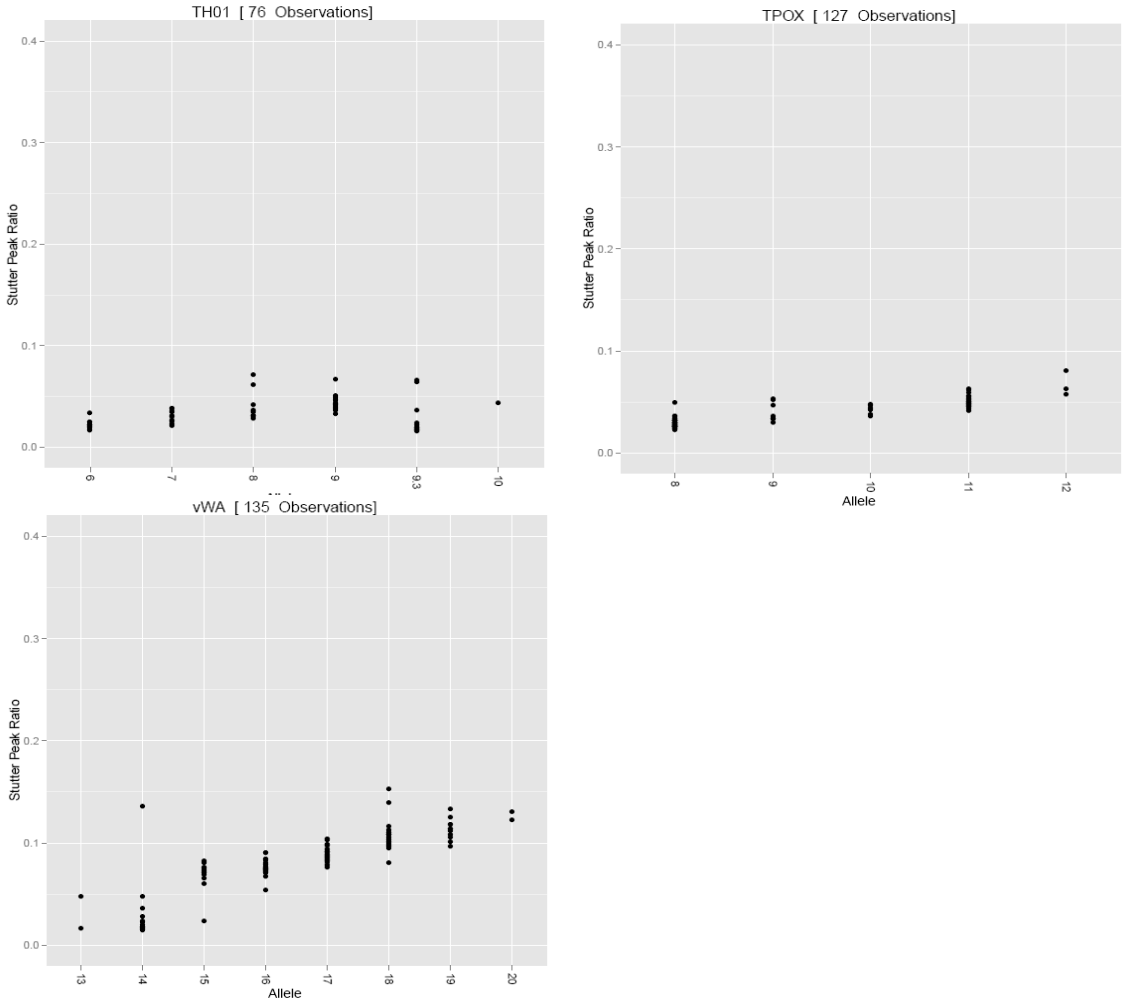


Figure 21. Stutter peak height ratios. Peak heights of forward stutters compared to main peaks were analyzed for 105 DNA samples from different donors.

Stutter peak heights are characteristic for each marker and the number of repeat motifs of an allele. TH01 in general shows the lowest stutter ratio of all STR markers of the Investigator IDplex Plus Kit. Smaller alleles display lower stutter levels than longer alleles of the same marker. Only forward stutter positions were analyzed, as backward stutters usually are of very low height, although present.

References

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Cited references

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3. Butler, J. (2006) Genetics and Genomics of Core Short Tandem Repeat Loci Used in Human Identity Testing. J. Forensic Sci. **51**, 253.

Ordering Information

Product	Contents	Cat. no.
Investigator IDplex Plus Kit (100)	Primer mix, Fast Reaction Mix including HotStarTaq® *Plus* DNA Polymerase, Control DNA, allelic ladder IDplex Plus, DNA size standard 550 (BTO), and nuclease-free water	381625
Investigator IDplex Plus Kit (400)	Primer mix, Fast Reaction Mix including HotStarTaq *Plus* DNA Polymerase, Control DNA, allelic ladder IDplex Plus, DNA size standard 550 (BTO), and nuclease-free water	381627

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