Validation Report December 2016

# Developmental validation of the Investigator® Argus X-12 QS Kit

The QIAGEN® Investigator Argus X-12 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 X-12 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 the 12 polymorphic X chromosomal STR markers DXS10103, DXS8378, DXS10101, DXS10134, DXS10074, DXS7132, DXS10135, DXS7423, DXS10146, DXS10079, HPRTB, and DXS10148, the gender-specific Amelogenin and the autosomal marker D21S11. The 12 X-chromosomal STR markers are clustered into four linkage groups (three markers per group; Table 1), and thus, each set of three markers is handled as a haplotype for genotyping. The markers used are described in numerous publications (for examples see references 3–8). The Forensic ChrX Research Group initiated the online data base ChrX-STR.org (www.chrx-str.org) that provides population data and haplotype frequencies. The autosomal marker D21S11 enables alignment of the Investigator Argus X-12 QS Kit profile with any other STR profile from the expanded CODIS or ESS standard set of markers to minimize the risk of a sample mix-up.

**Table 1. Linkage groups**

| Linkage group | |
|---|---|
| 1 (Xp22) | DXS8378 – DXS10135 – DXS10148 |
| 2 (Xp11) | DXS7132 – DXS10074 – DXS10079 |
| 3 (Xp26) | HPRTB – DXS10101 – DXS10103 |
| 4 (Xp28) | DXS7423 – DXS10134 – DXS10146 |

The Investigator Argus X-12 QS Kit contains an internal PCR control (Quality Sensor™ QS1), which provides helpful information about the efficiency of the PCR. 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, please refer to the *Investigator Argus X-12 QS Kit Handbook*.

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 effect of variations in buffer component concentrations is described using, as an example, $MgCl_2$ concentration (page 3). 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).

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 17). Cross-reactivity with non-human DNA (page 19) was also assessed. The reproducibility of the results was verified (page 23).

## 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. The standard conditions specified in the *Investigator Argus X-12 QS Kit Handbook* were used for electrophoresis. Unless stated otherwise, a GeneAmp® PCR System 9700 with a Gold-plated Silver 96-Well Block was used for amplification. Data were analyzed using 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 2.0 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 ±30% of the optimum.

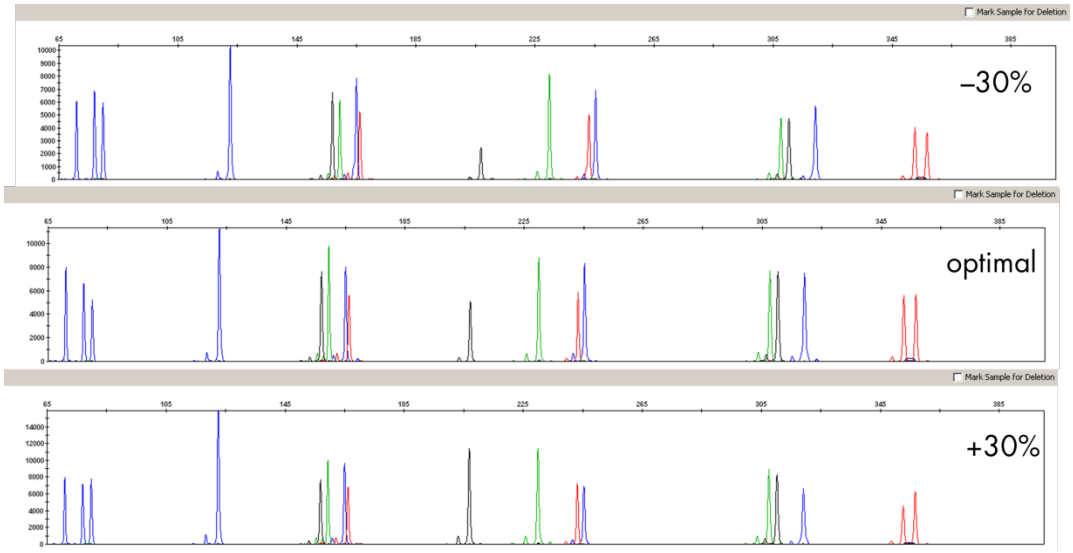



**Figure 1. Influence of $MgCl_2$ concentration.** Fast Reaction Mix 2.0 (FRM 2.0) was supplemented with different concentrations of $MgCl_2$ matching the specification of production, or 30% higher and lower. Representative electropherograms of sample quadruplicates are shown.

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).

## Effect of PCR annealing temperature variations

Specificity, sensitivity and robustness are critical in forensic casework, all of which are affected by the annealing temperature ($T_m$). 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 X-12 QS Kit reaction.

**Table 2. Standard cycling protocol recommended for all DNA samples**

| Temperature | Time | Number of cycles |
|---|---|---|
| 98°C* | 60 s | 3 cycles |
| 61°C | 100 s | |
| 72°C | 5 s | |
| 96°C | 10 s | 27 cycles |
| 61°C | 100 s | |
| 72°C | 5 s | |
| 68°C | 2 min | |
| 10°C | ∞ | – |

* Hot-start to activate DNA polymerase.

Annealing temperatures between –4°C and +4°C around the optimal annealing temperature of 61°C were applied to the amplification of 500 pg control DNA 9947. PCR was performed on an Eppendorf® Mastercycler® ep instrument. Reactions using annealing temperatures between –4°C and +3°C resulted in full profiles. Good overall inter-locus balance was observed for the temperature range of –3.5°C to +2.0°C, with only marker DXS10146 showing reduced peak height at +2.0°C (Figure 2 and Figure 3). First dropouts were observed at +3.5°C above the recommended temperature applying a threshold of 200 RFU. No non-specific PCR products were observed. 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).

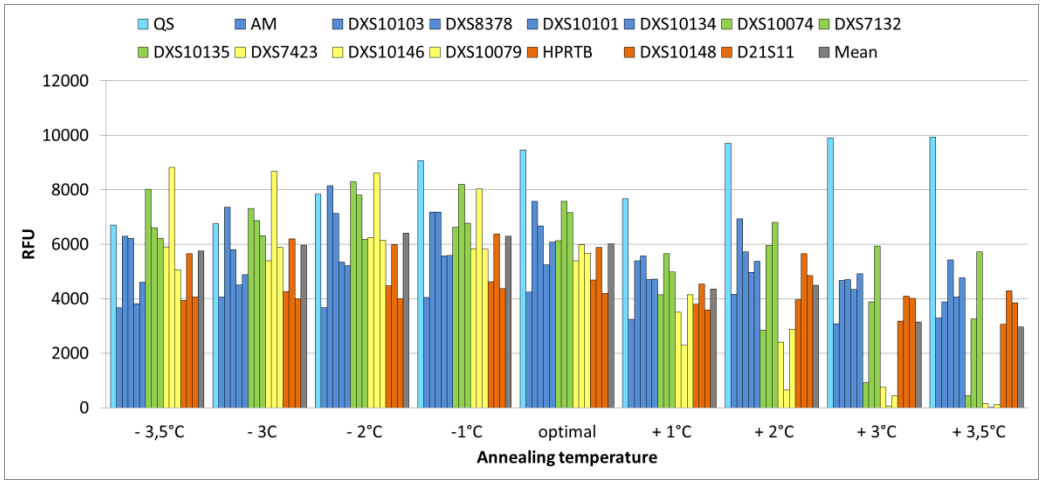



**Figure 2. Effect of variations in the PCR annealing temperature.** 500 pg control DNA 9947 was amplified on an Eppendorf Mastercycler ep instrument, under standard conditions (30 cycles). Each bar represents an average peak height of triplicates. Bar colors represent the fluorescent dye labels of individual markers, gray bars show the average of all STR markers.

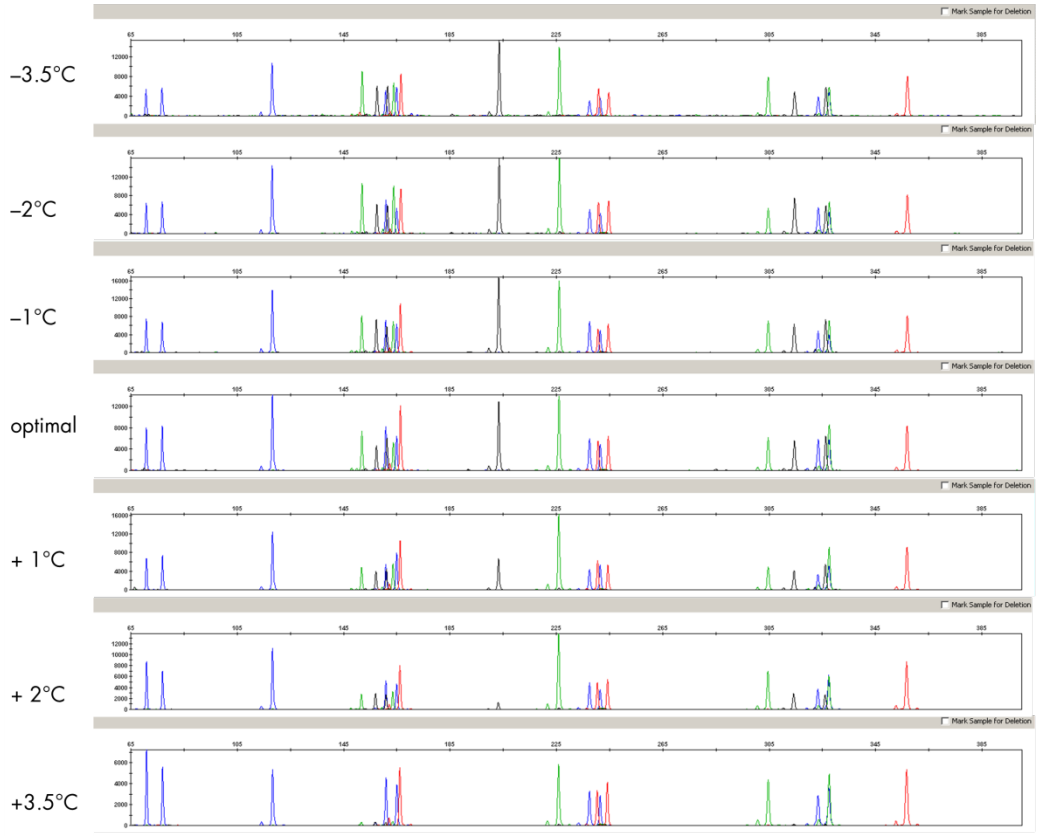



**Figure 3. Variations in the PCR annealing temperature.** 500 pg control DNA 9947 was amplified on an Eppendorf Mastercycler ep. Representative electropherograms for reactions using annealing temperatures between –3.5°C and +3.5°C are shown. Note that the y-axis was scaled for best fit.

## 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 speed up the protocol when the DNA sample is abundant (e.g., for database samples).

Cycle numbers were increased to 32 or 34 for reactions containing 32, 16 or 8 pg of control DNA 9947, and the numbers of called alleles, as well as peak heights, were compared to a standard 30-cycle protocol (Figure 4). Here, the cycle numbers of the second cycling block were increased from 27 cycles to 29 or 31, while the first 3 cycles of the standard protocol were not changed. As expected, signal intensities of amplified products increased with higher cycle numbers. However, it should be noted that an increase in overall 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 of 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 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.

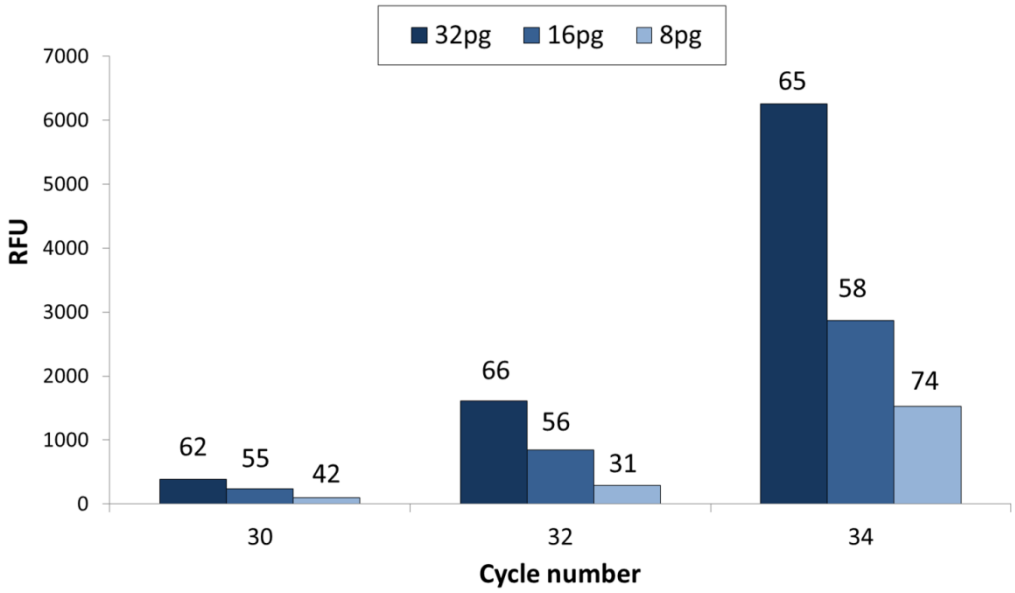



**Figure 4. Effect of different cycle numbers on mean peak height and number of called alleles.** Different amounts of control DNA 9947 were used as template, as indicated in the figure. Samples were run in triplicates and numbers of detected PCR products (indicated above the bars; 99 PCR products expected) and their peak heights were 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 of template DNA (Figure 5). Here, the cycle numbers of the second cycling block were decreased from 27 to 21, 23 or 25, while the first 3 cycles of the standard protocol were not changed. As expected, all reactions resulted in robust amplification and full profiles were obtained using a threshold of 50 RFU for allele calling. However, amplifications with a total of 28 total cycles, using 10 ng template DNA, gave rise to "pull up" peaks when applying samples to analysis without prior dilution of the PCR product. An example electropherogram for amplification with 24 cycles in total, using 2.5 ng template DNA, is shown in Figure 6. Please note, when reducing the cycle number, the peak height of the Quality Sensor QS1 decreases and may drop below the threshold. The template amount of the Quality Sensor is optimized for 30 cycles; thus reducing the cycle number will reduce the QS signal.

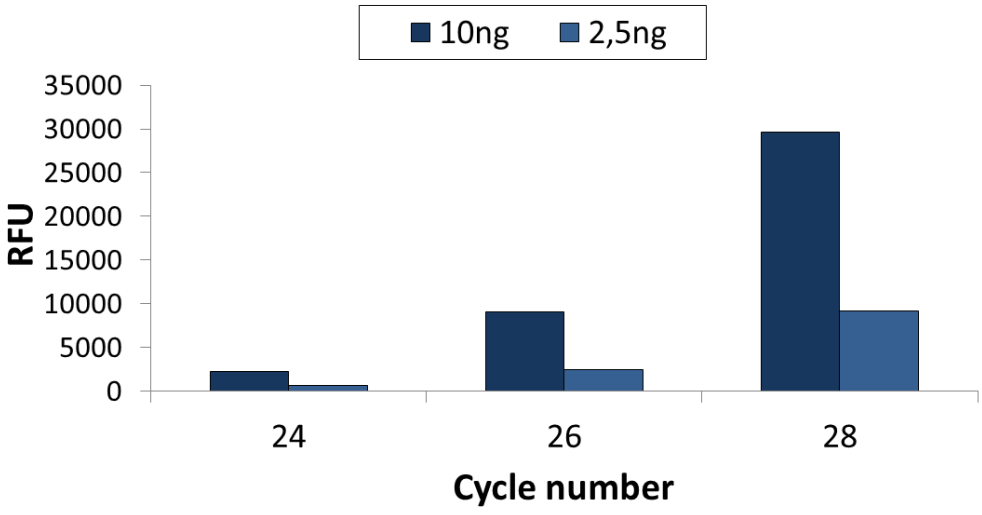



**Figure 5. Effect of decreased cycle numbers on mean peak heights.** 2.5 ng or 10 ng of control DNA 9948 were subjected to amplification using a total of 24, 26 or 28 PCR cycles. Samples were run in triplicates and average peak heights calculated.

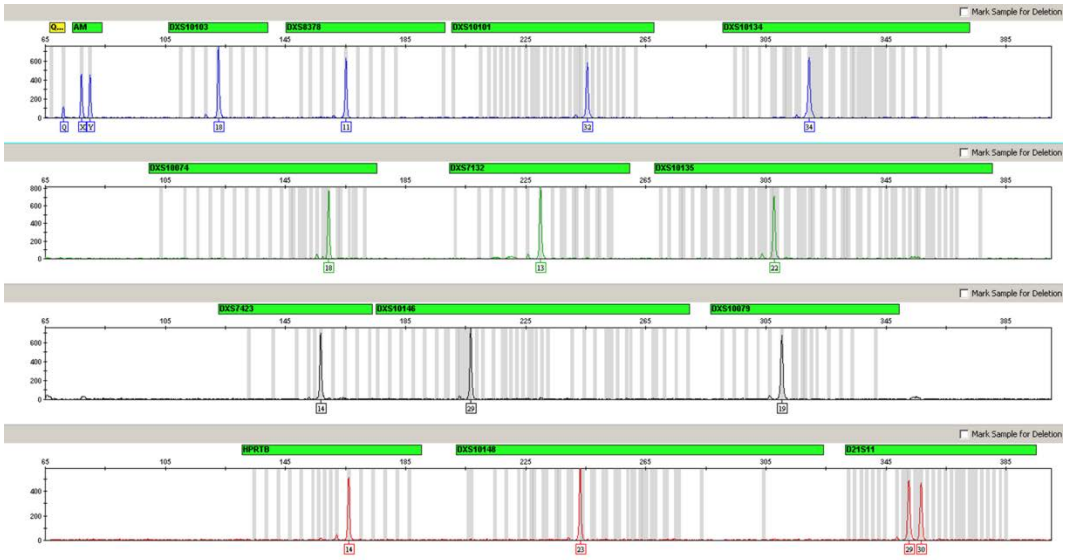


**Figure 6. Reduction of PCR cycle numbers with elevated template DNA amounts.** Results for amplification of 2.5 ng control DNA 9948, using a total of 24 instead of the standard 30 PCR cycles. The template amount of the Quality Sensor is optimized for 30 cycles, thus reducing the cycle number reduces the QS signals detected.

## Effect of different cycler types

Several PCR thermal cyclers were tested with the Investigator Argus X-12 QS Kit, to demonstrate kit robustness independent of the instrument (Figure 7). 2 ng to 32 pg of control DNA 9948 were

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)
- Bio-Rad® C1000 Touch Thermal Cycler (Bio-Rad Laboratories GmbH, Munich, Germany)
- Veriti® 96-Well Thermal Cycler (Applied Biosystems Inc., Foster City, CA, USA)
- Eppendorf Mastercycler ep (Eppendorf AG, Hamburg, Germany)
- MJ Research DNA Engine PTC-200 Peltier Thermal Cycler (Bio-Rad Laboratories GmbH, Munich, Germany)

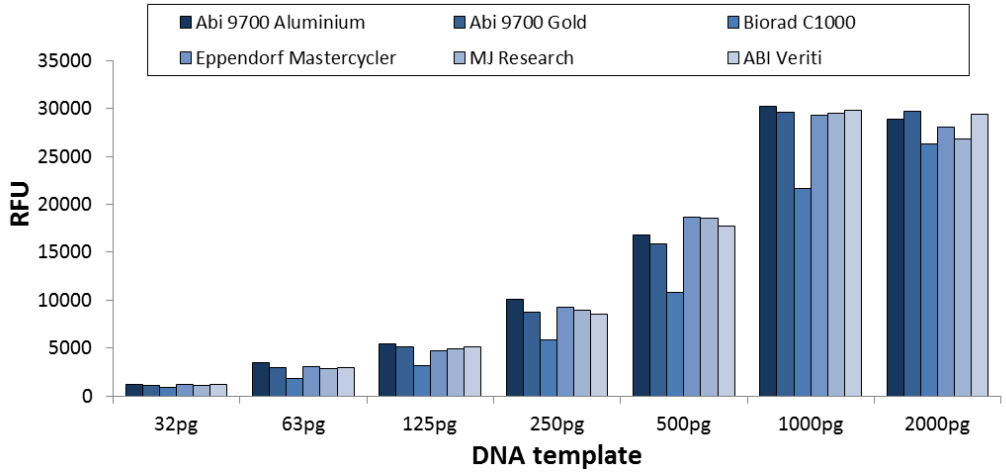



**Figure 7. Performance on different PCR thermal cyclers.** Different amounts of control DNA 9948 were used as, as indicated in the figure. Each sample was run in duplicate. A standard 30-cycle protocol was used. Average peak heights across all markers are shown.

Similar mean peak heights were obtained for all of the tested PCR cyclers; the highest difference was observed for the Bio-Rad C1000 showing about 30% reduced peak heights. No significant differences in intra- and inter-locus balance or non-specific amplification was observed on any of the thermal cyclers.

## Effect of different genetic analyzers

The Investigator Argus X-12 QS Kit uses a five color setup with fluorescence-labeled primers with the following dyes 6-FAM™, BTG, BTY, BTR, and the BTO labeled size standard. Two Applied Biosystems 3500 Genetic Analyzers and the Applied Biosystems 3130 Genetic Analyzer at different locations were tested in order to demonstrate the robustness of the Investigator Argus X-

12 QS Kit. The data shown in Figure 8 were generated using aliquots of the same PCR products. 500 pg of control DNA 9948 and control DNA 9947 were each amplified in duplicate. The reaction took place under standard conditions.

Variations reflected differences in sensitivity between individual instruments, for example, due to laser power, array life and matrix calibration effects. Limitations of genetic analyzer sensitivity must be addressed by individual laboratories during an internal validation study, using the instrumentation in their laboratory.

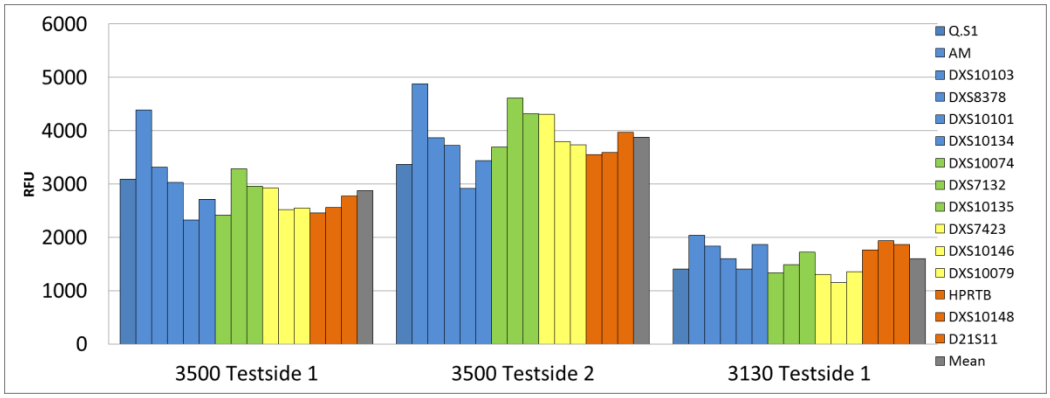



**Figure 8. Analysis of PCR products on different Applied Biosystems Genetic Analyzers.** 500 pg of control DNA 9948 and 9947 were amplified on a GeneAmp PCR System 9700, in duplicate. Samples were run according to the *Investigator Argus X-12 QS Kit Handbook*. Aliquots of the same PCR were delivered to three different test sites and analyzed on Applied Biosystems 3500 or 3130 Genetic Analyzers. Average peak heights for duplicates of both DNAs are shown.

## Sensitivity

The Investigator Argus X-12 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 Kits. In particular, for heavily degraded DNA, the use of increased template amounts may improve results (see page 17).

Control DNA 9947 was serially diluted from 500 pg to 8 pg per reaction. Full profiles (31 PCR products) were consistently obtained at 125 pg, using the standard conditions specified in the *Investigator Argus X-12 QS Kit Handbook*. Occasional allele dropouts were found due to stochastic effects when ≤63 pg DNA was used as 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 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.

"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. Figure 9 and Figure 10 show 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.

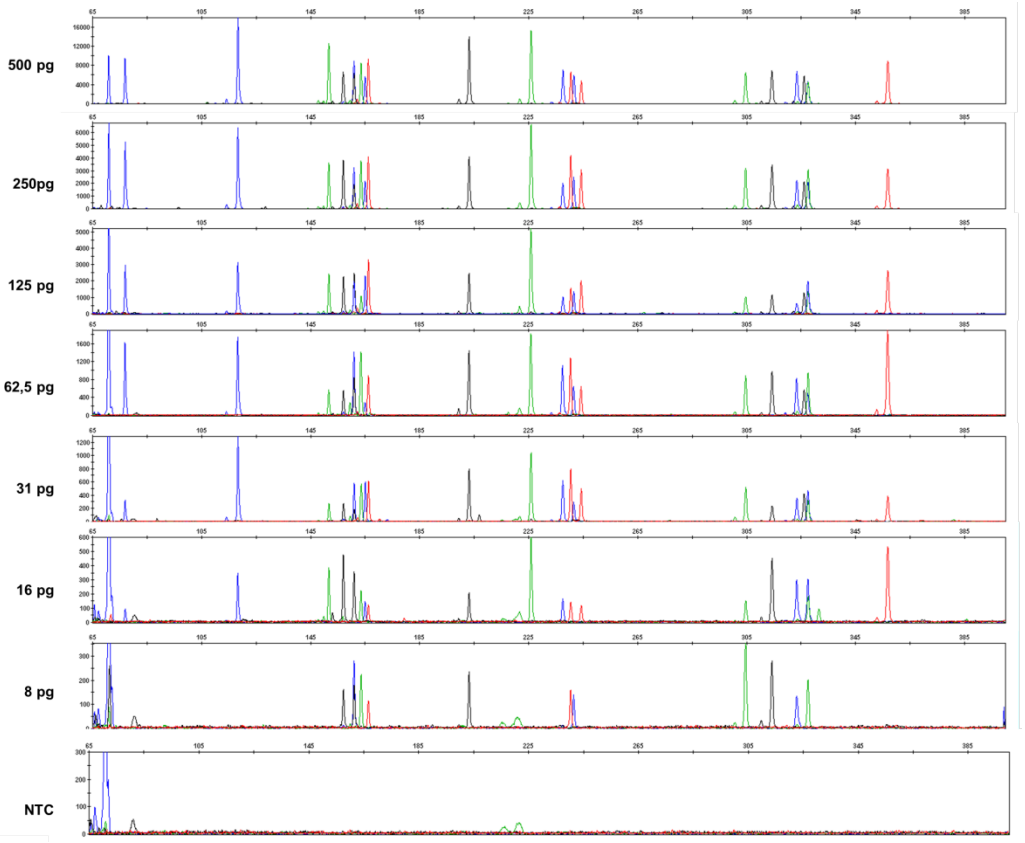



**Figure 9. Sensitivity study.** Serial dilutions of control DNA 9947 were analyzed. The amounts of DNA indicated were used as template for amplification. The lowest panels show a no-template (negative) control (NTC) to illustrate the expected level of background fluorescence. Y-axis scales were adjusted individually for the highest peak heights of the STR markers. Note: As the Quality Sensor (first blue peak) is amplified with similar amplification efficiency in all experiments independently of the sample template amounts, the QS peak heights are similar in all experiments.

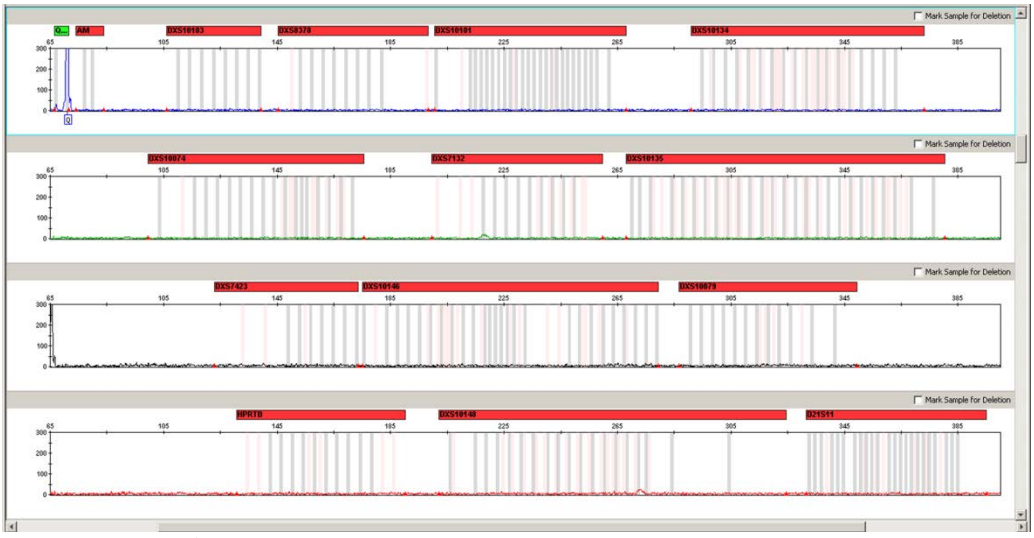


**Figure 10. Background fluorescence.** Example of a no-template (negative) control to illustrate the expected level of background fluorescence. For correct interpretation of samples having only small amounts of DNA, it is crucial to minimize background fluorescence

## Performance with simulated inhibition

If the DNA extraction from forensic casework samples is done using inappropriate methods, Investigator Argus X-12 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.

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.

Results are shown in Figures 11–16. Figure 17 shows an overview of Investigator Argus X-12 QS Kit inhibitor resistance.

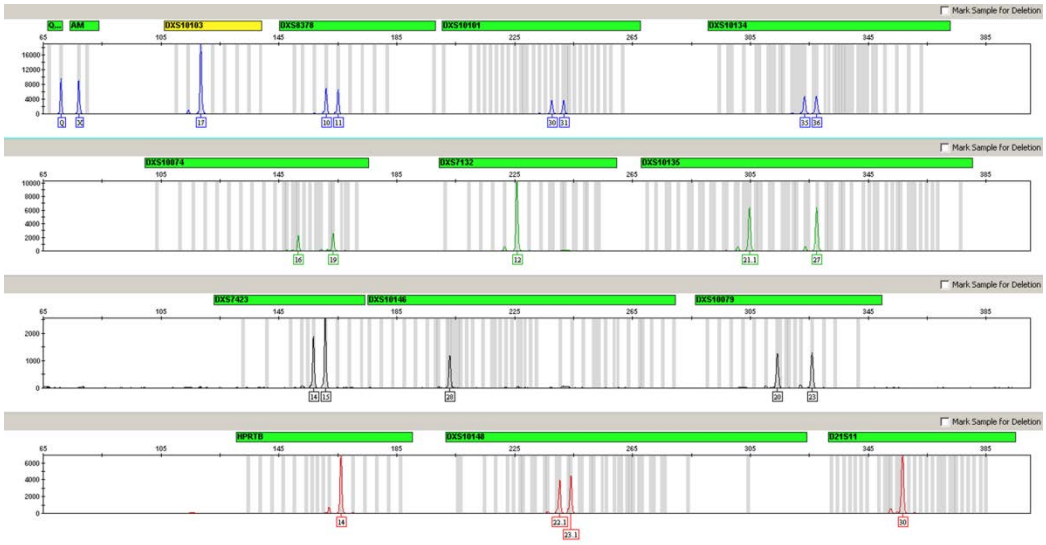



**Figure 11. Electropherogram of 500 pg control DNA 9947 amplified in the presence of 500 µM hematin.**

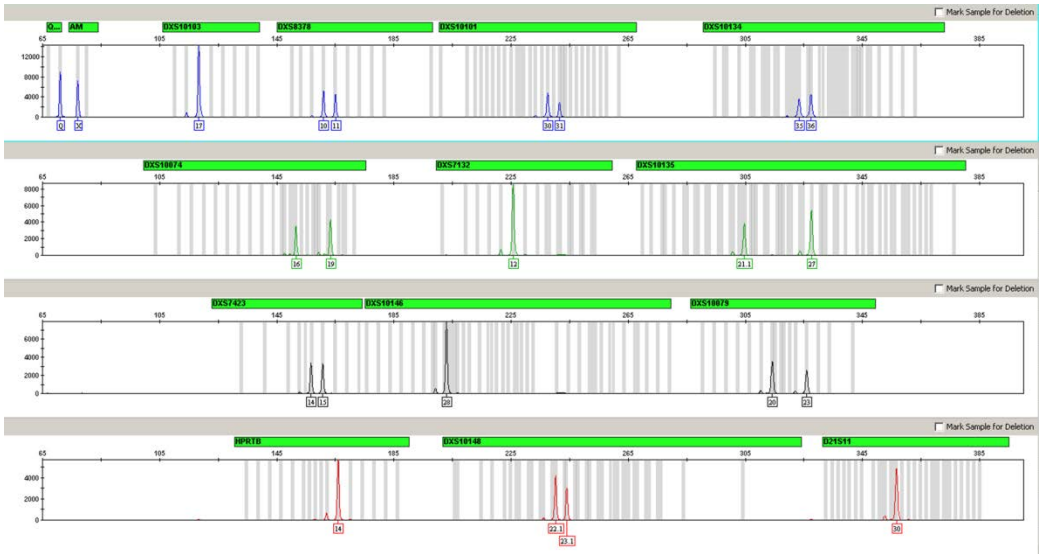



**Figure 12. Electropherogram of 500 pg control DNA 9947 amplified in the presence of 200 ng/µl humic acid.**

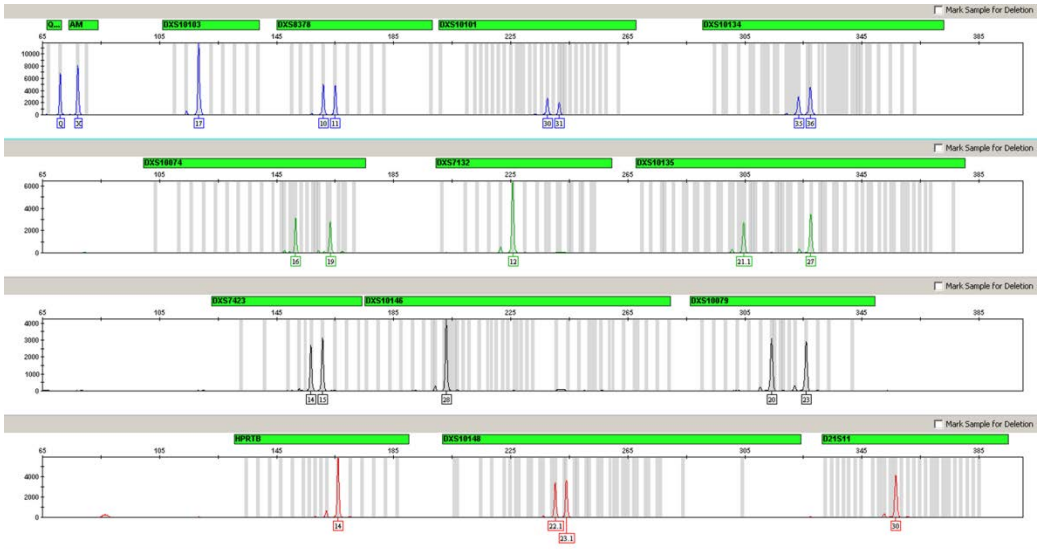



**Figure 13. Electropherogram of 500 pg Control DNA 9947 amplified in the presence of 6 mM indigo carmine.**

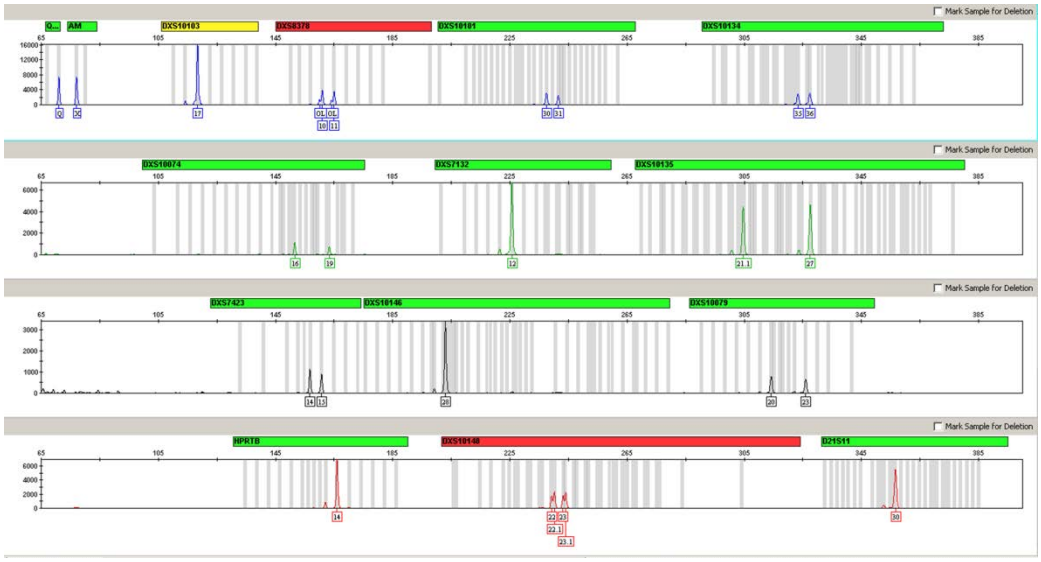



**Figure 14. Electropherogram of 500 pg control DNA 9947 amplified in the presence of 150 ng/µl collagen.**

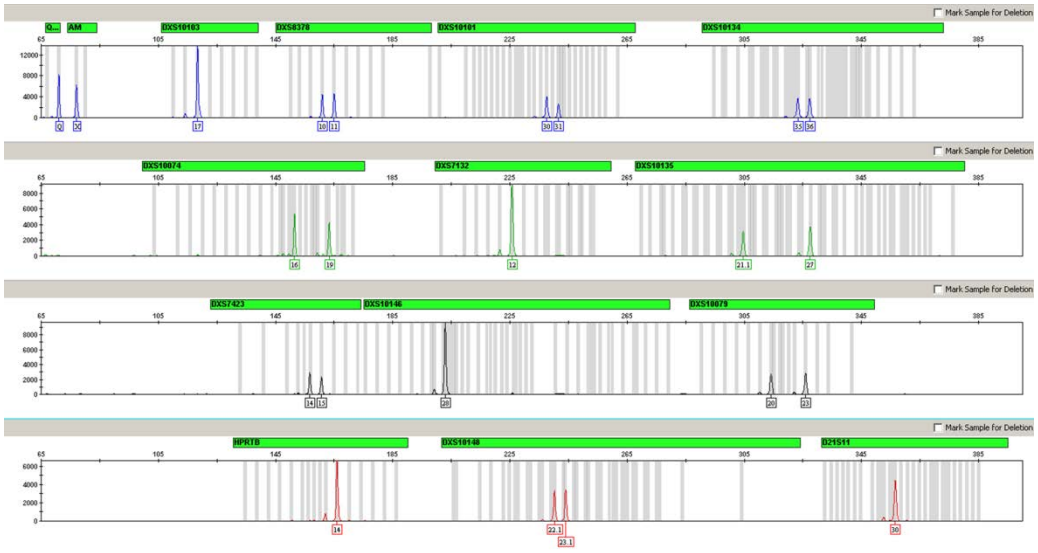



**Figure 15. Electropherogram of 500 pg control DNA 9947 amplified in the presence of 2 mM calcium.**

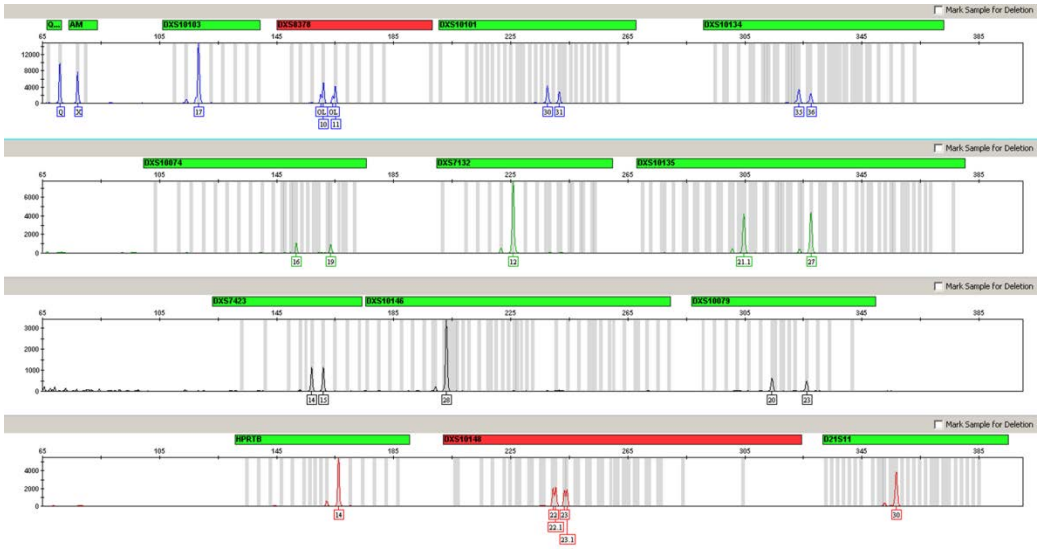



**Figure 16. Electropherogram of 500 pg Control DNA 9947 amplified in the presence of 3000 ng/µl tannic acid.**

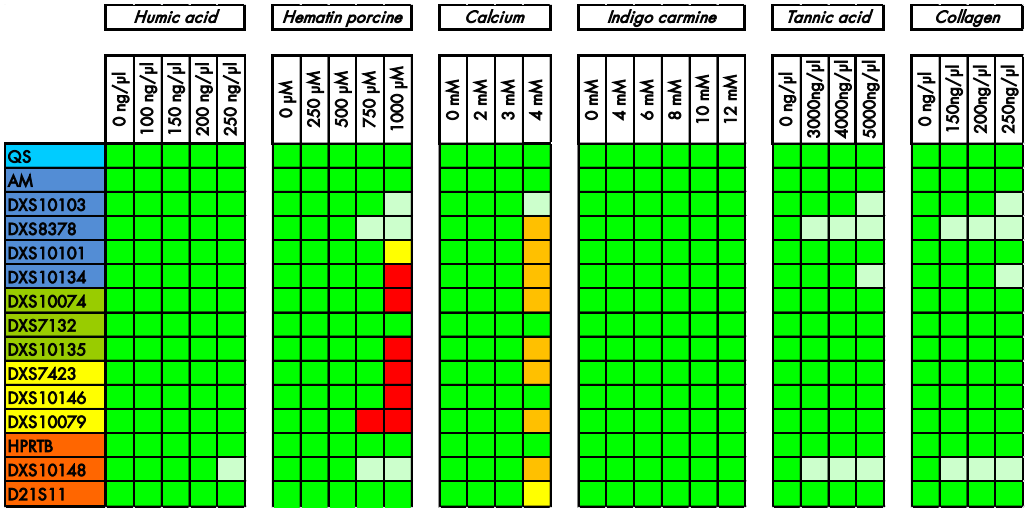



**Figure 17. Overview of Investigator Argus X-12 QS inhibitor resistance.** The assay was tested for its robustness towards inhibitors (humic acid, hematin, tannic acid, indigo carmine, calcium, and collagen). 500 pg of control DNA 9947 was used as template and PCR was performed under standard conditions. 50 RFU was used as a 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. Collagen shows a different mode of interference with PCR amplification by affecting 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 14). This effect is sequence dependent and therefore 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.

## 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 these samples may therefore show varying degrees of degradation.

Artificially degraded male genomic DNA 9948 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. Fragment lengths were verified on an agarose gel and 500 pg DNA was used as template for

amplification (all samples in triplicate) (Figure 18A). 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. At an average fragment length of 150 bp, 37 out of 48 peaks (77%) to be expected in triplicate samples were detected using a threshold of 50 RFU (Figure 18B). Increased amounts of template (up to 2 ng), can be used to improve results for heavily degraded DNA.

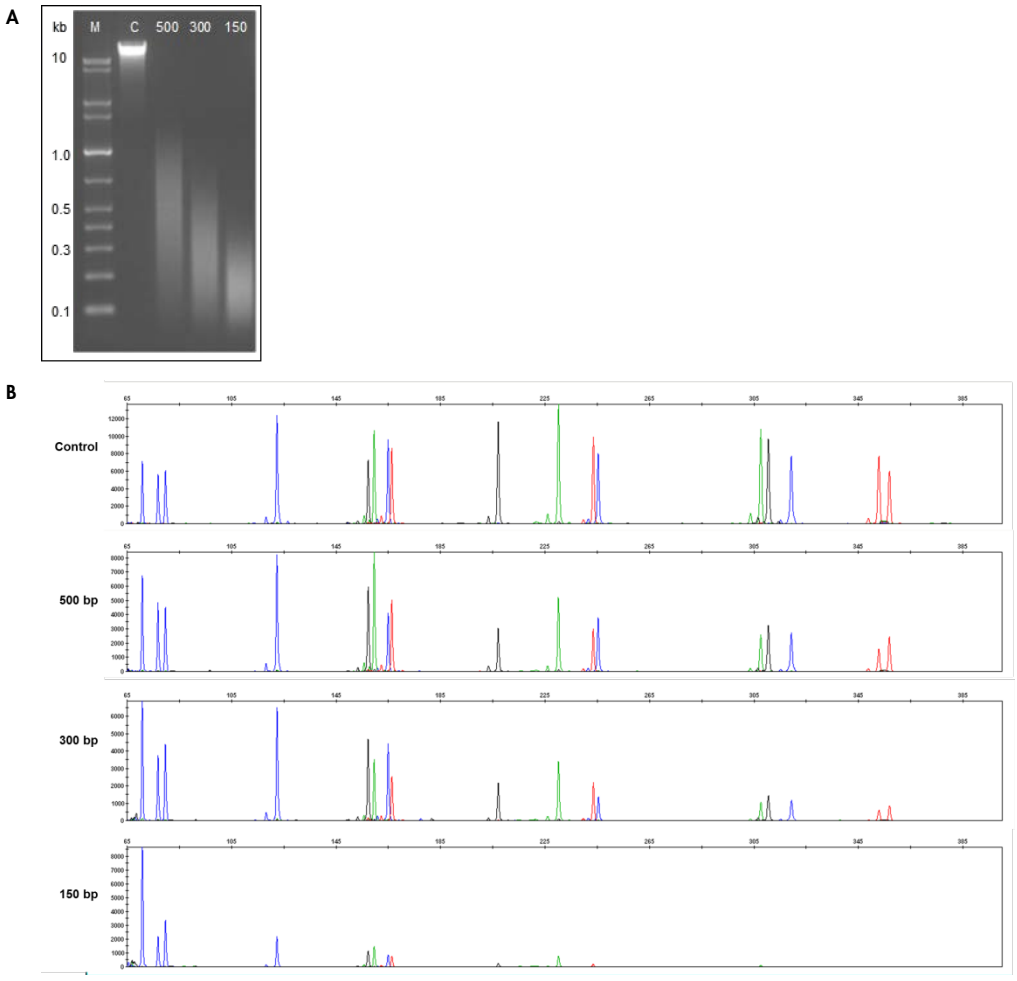



**Figure 18. Degraded DNA.** Male DNA 9948 was sheared to the defined average fragment length indicated. **A**: Agarose gel analysis of degraded DNA. **M**: DNA size standard markers, **C**: Untreated DNA. 500, 300, 150: average fragment lengths in base pairs. **B**: STR analysis of untreated control and degraded DNA samples. 500 pg template DNA was used. Full profiles were obtained down to 300 bp average fragment length.

## 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 X-12 QS Kit species specificity for human DNA, DNA from other species was tested following the standard assay protocol.

Besides common pets and farm animals, some primates were also tested. As expected for primates, amplification of some products is possible. Chimpanzees, bonobos, orangutans and gorillas give rise to several peaks within marker ranges in all channels, some of which match the size of human STR products. Dog DNA gives rise to major peaks outside the marker range of the BTY channel at about 350–400 bp that seem to be derived from STR markers and reproducible peaks at 123 bp and 165 bp. Other animals showed low level cross-reactivity with no reproducible peaks (Figure 19).

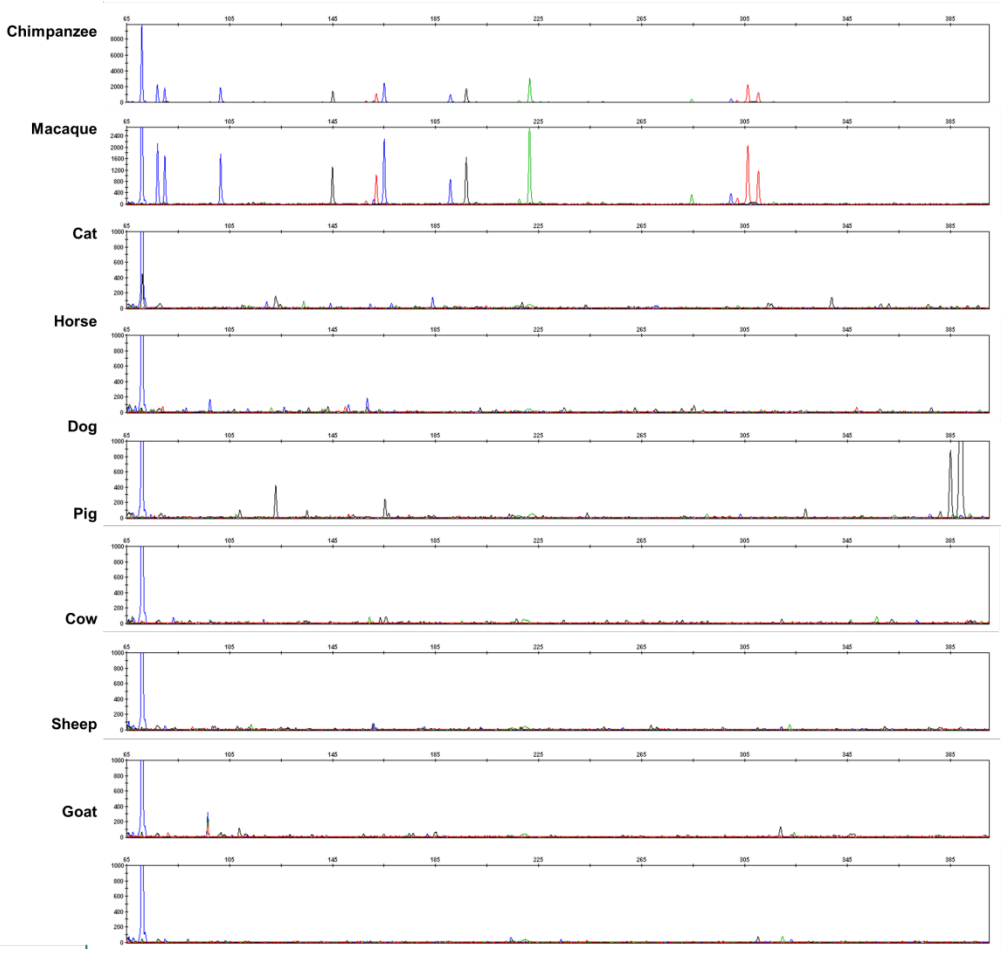



**Figure 19. Representative results of the species specificity assessment.** 500 pg primate DNA and 2.5 ng DNA from all other species was used as template.

## 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 9947 and XX11 in ratios of 1:1, 3:1, 7:1, 10:1, 15:1 and vice versa (see Table 4 for genotypes of 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 DNA and 469 pg of the major component (Table 3). 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 1:10 mixtures is shown in Figure 20. 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.

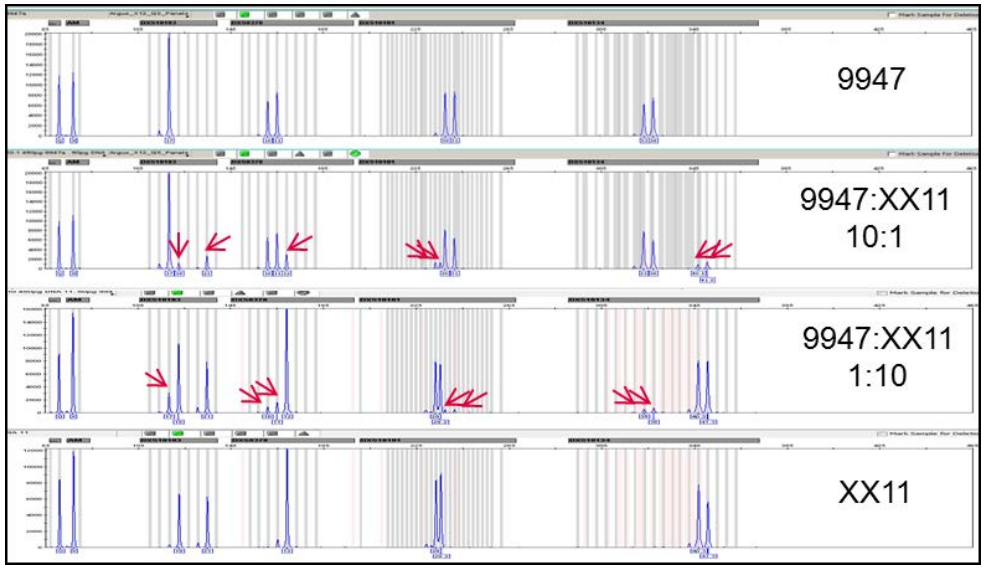
**Table 3. Amounts of DNA template in mixtures**

| Mixture ratio | Major component | Minor component |
|---|---|---|
| 1:1 | 250 pg | 250 pg |
| 3:1 | 375 pg | 125 pg |
| 7:1 | 429 pg | 71 pg |
| 10:1 | 450 pg | 50 pg |
| 15:1 | 467 pg | 33 pg |

**Table 4. Genotypes of DNAs used for mixed samples**

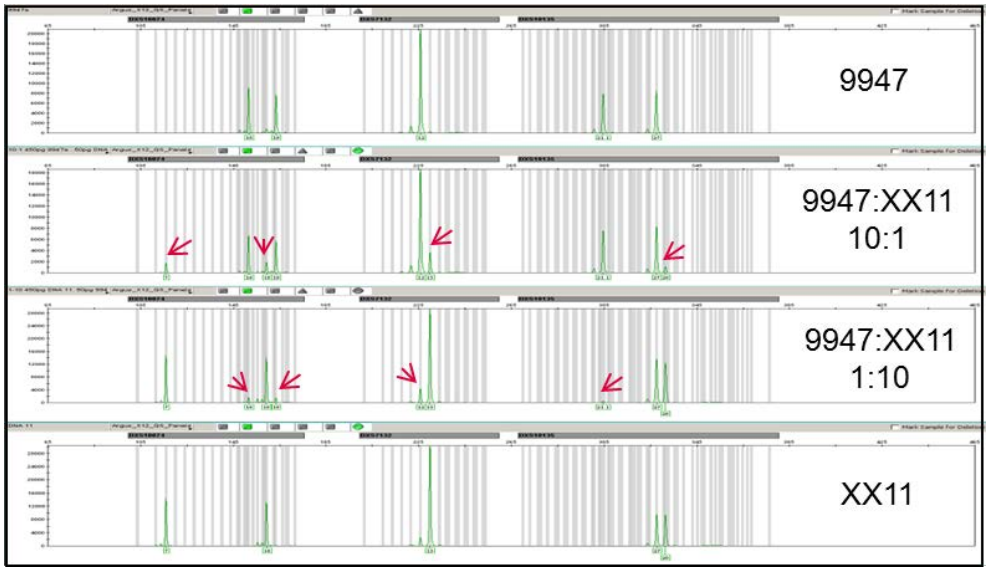
| Locus | 9947 genotype | XX11 genotype |
|---|---|---|
| Amelogenin | X/X | X/X |
| DXS7132 | 12/12 | 13/13 |
| DXS7423 | 14/15 | 14/15 |
| DXS8378 | 10/11 | 12/12 |
| DXS10074 | 16/19 | 7/18 |
| DXS10079 | 20/23 | 15/21 |
| DXS10101 | 30/31 | 29/29.2 |
| DXS10103 | 17/17 | 18/21 |
| DXS10134 | 35/36 | 40.3/41.3 |
| DXS10135 | 21.1/27 | 27/28 |
| DXS10146 | 28/28 | 23/39.2 |
| DXS10148 | 22.1/23.1 | 26.1/29.1 |
| HPRTB | 14/14 | 12/15 |
| D21S11 | 30/30 | 30/33.2 |

**A**




See below for general figure legend.
Expected bp positions of uncovered minor component peaks are indicated by red arrows. Amelogenin: Both samples are female, no minor component allele. DXS10103: Homozygote + heterozygote, no overlapping alleles, three minor component peaks can be assigned. DXS8378: Homozygote + heterozygote, no overlapping alleles, two minor component peaks can be assigned, one minor component peak is overlapping with stutter position. DXS10101: Heterozygote + heterozygote, no overlapping alleles, three minor component peaks can be assigned, one minor component peak is overlapping with stutter position. DXS10134: Heterozygote + heterozygote, no overlapping alleles, all minor component alleles can be assigned.

B

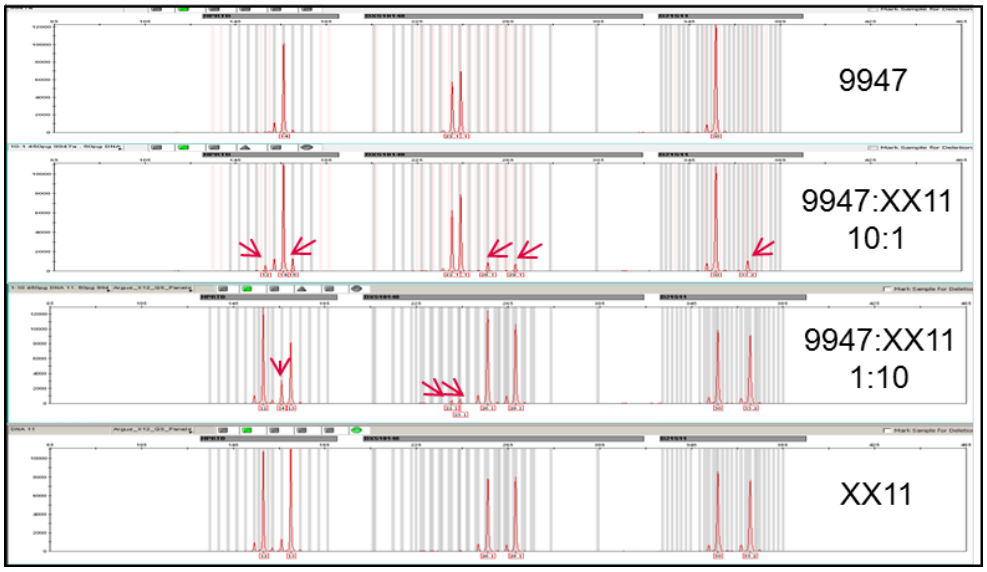



See below for general figure legend.
Expected bp positions of uncovered minor component peaks are indicated by red arrows. DXS10074: Heterozygote + heterozygote, no overlapping alleles, one minor component peak is overlapping with stutter position, three minor component peaks can be assigned. DXS7132: Homozygote + homozygote, one minor component peak is overlapping with stutter position, the non-overlapping minor component peak can be assigned. DXS10135: Heterozygote + heterozygote, one overlapping allele, two non-overlapping minor component peaks can be assigned.

C


See below for general figure legend.
Expected bp positions of uncovered minor component peaks are indicated by red arrows. DXS7423: Heterozygote + heterozygote, all alleles overlap. DXS10146: Heterozygote + homozygote, no overlapping alleles, all non-overlapping minor component peaks can be assigned. DXS10079: Heterozygote + heterozygote, no overlapping alleles, one minor component peak is overlapping with stutter position, two non-overlapping minor component peak can be assigned.

D




See below for general figure legend.
Expected bp positions of uncovered minor component peaks are indicated by red arrows. HPRTB: Heterozygote + homozygote, no overlapping allele, one minor component peak is overlapping with stutter position, the two non-overlapping minor component peaks can be assigned. DXS10148: Heterozygote + heterozygote, no overlapping allele, all four minor component peaks can be assigned. D21S11: Heterozygote + homozygote, one overlapping allele, one minor component peak can be assigned.

**Figure 20. Results of the mixture analysis.** Figures A–D show 1:10 mixtures of control DNA 9947 and XX11 (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, is to be expected. A brief description of the mixture situation given for each individual marker is given below the figures.

## Reproducibility: Concordance test

The primer sequences of the Investigator Argus X-12 QS Kit haves been adopted from the parent kit Investigator Argus X-12. The primer sequences for the alignment marker D21S11 are identical to those used in autosomal Investigator kits. Sequence variants of DXS10101, DXS10146 and DXS10148 have been reported to occur at an elevated frequency in African populations (9). The corresponding mutations affected primer binding and have been addressed by introducing additional SNP primers to the Argus X-12 QS primer mix compared to earlier X-chromosomal commercially available STR Kits for human identification and paternity analysis. A concordance study was performed on a set of 123 samples of mainly Caucasian origin. The genotypes obtained for the X-markers were all identical between the two generations of Argus X-12 kits.

Peak height ratios of heterozygous markers (Figure 21) and stutter peak heights (Figure 22) were analyzed based on data from the concordance study.

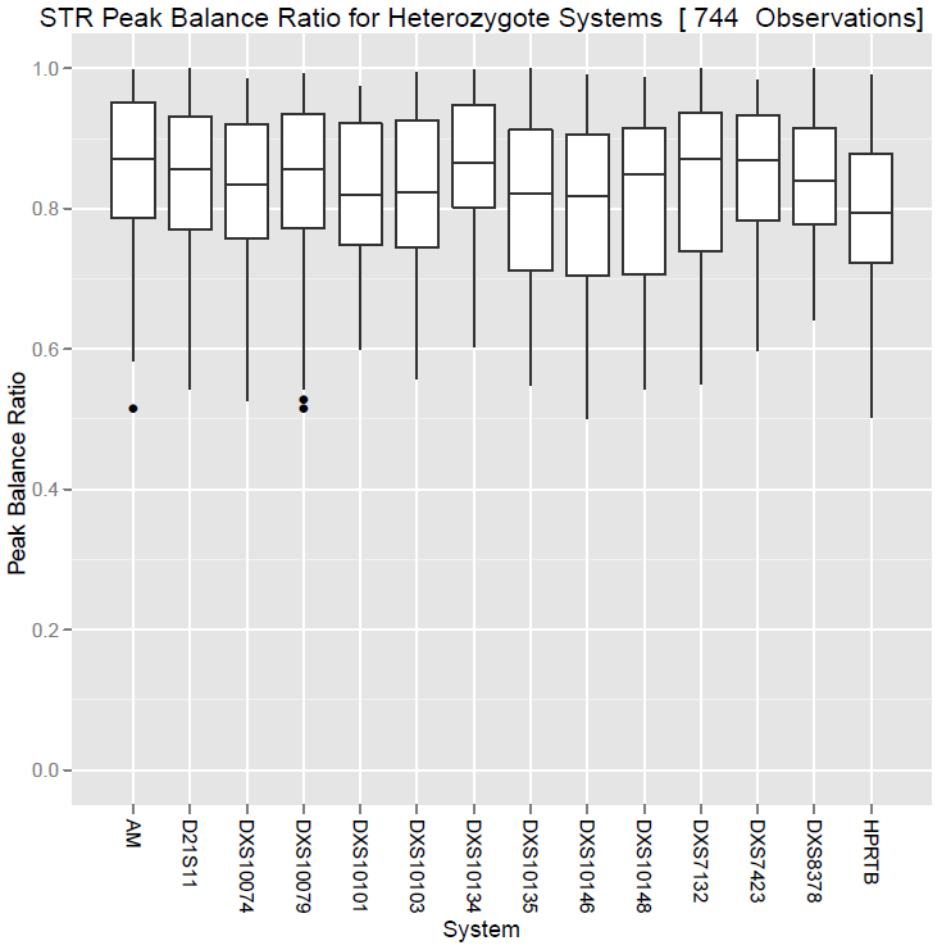
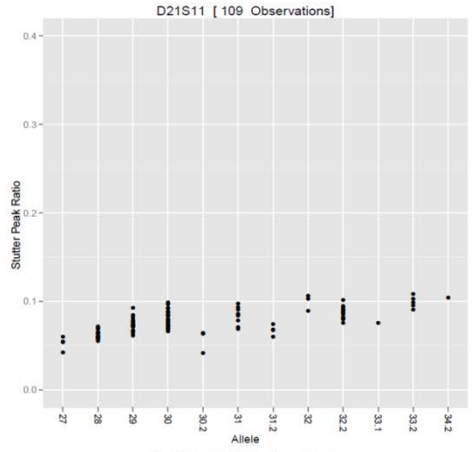
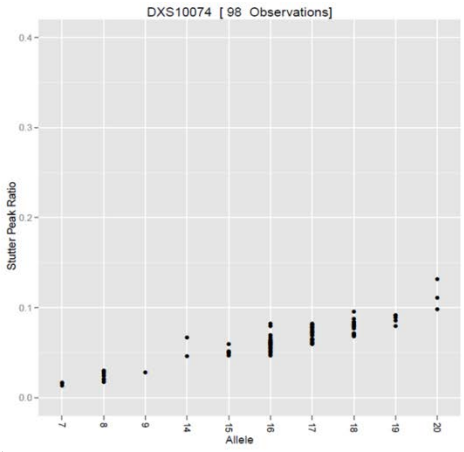
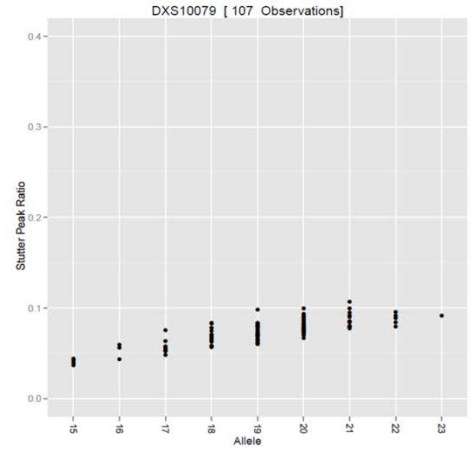
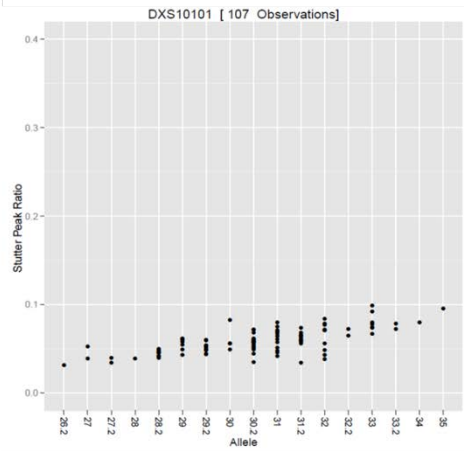


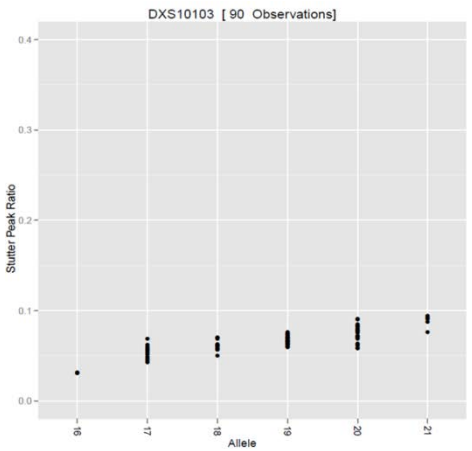

**Figure 21. Box plot analysis of intra-locus peak height ratios.** Values refer to the lower of the 2 peaks in a heterozygous sample. Boxes represent the middle 50% (interquartile range, [IQR]) of data, lines inside the boxes the median. Whiskers show data within 1.5 IQR, dots represent outliers.

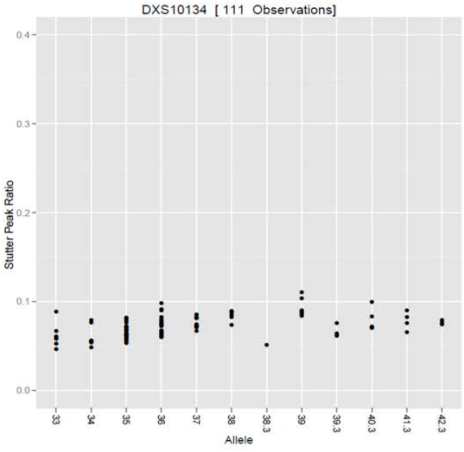

D21S11 [ 109 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
27
28
29
30
30.2
31
31.2
32
32.2
33.1
33.2
34.2
Allele

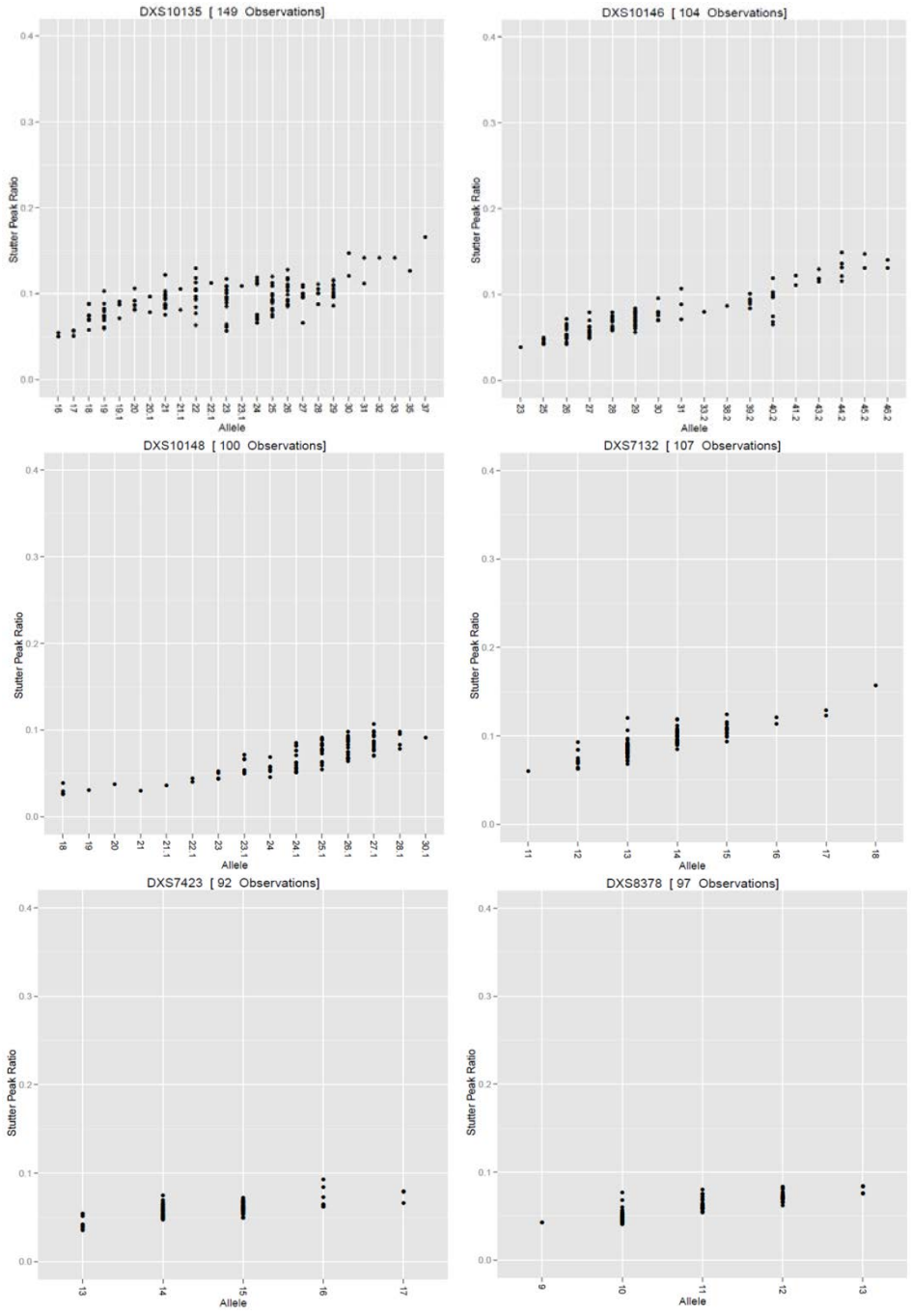

DXS10074 [ 98 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
7
8
9
14
15
16
17
18
19
20
Allele


DXS10079 [ 107 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
15
16
17
18
19
20
21
22
23
Allele


DXS10101 [ 107 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
26.2
27
27.2
28
28.2
29
29.2
30
30.2
31
31.2
32
32.2
33
33.2
34
35
Allele


DXS10103 [ 90 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
16
17
18
19
20
21
Allele


DXS10134 [ 111 Observations]
Stutter Peak Ratio
0.0
0.1
0.2
0.3
0.4
33
34
35
36
37
38
38.3
39
39.3
40.3
41.3
42.3
Allele


DXS10135 [ 149 Observations]
DXS10146 [ 104 Observations]
DXS10148 [ 100 Observations]
DXS7132 [ 107 Observations]
DXS7423 [ 92 Observations]
DXS8378 [ 97 Observations]
Stutter Peak Ratio
Allele

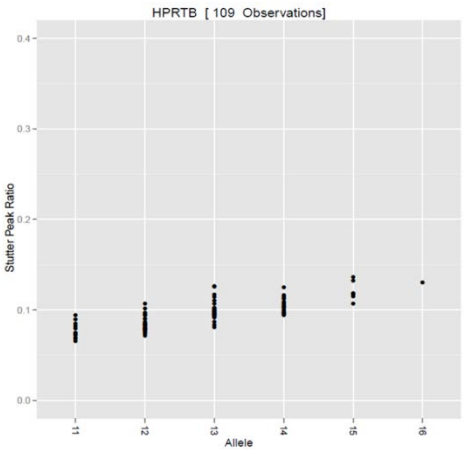



**Figure 22. Stutter peak height ratios.** Peak heights of forward stutters compared to main peaks were analyzed for 123 DNA samples from different donors.

## Stability – Direct amplification

The Investigator Argus X-12 QS Kit allows direct amplification of typical reference samples, such as blood or buccal cells on FTA, or buccal swabs. For amplification of buccal swabs we recommend to prepare a crude lysate using the Investigator STR Lysis Buffer. 2 µl lysate can be applied as template for amplification. For FTA based samples, one 1.2 mm punch can be used and Investigator STR GO! Punch Buffer should be added for amplification. The standard cycling protocol typically has to be adapted by reducing PCR cycle numbers. We recommend optimizing cycle numbers based on a representative batch of samples. Note that the Quality Sensor is optimized for 30 cycle amplifications and thus will show reduced signal heights at lower cycle numbers (see Figure 23 and Figure 24).

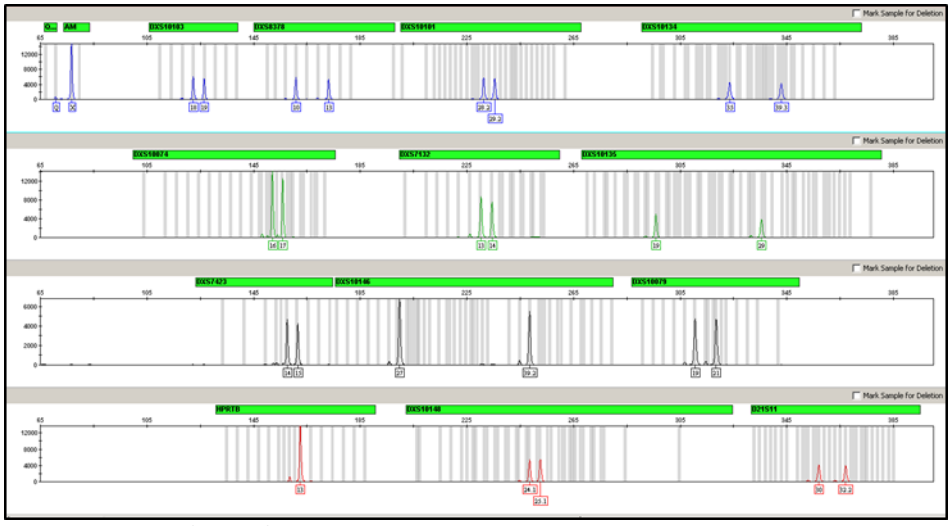


**Figure 23. Direct amplification of buccal cells on FTA.** A 1.2 mm punch was taken from an easiCollect Card (GE Healthcare) and amplified in 27 PCR cycles.

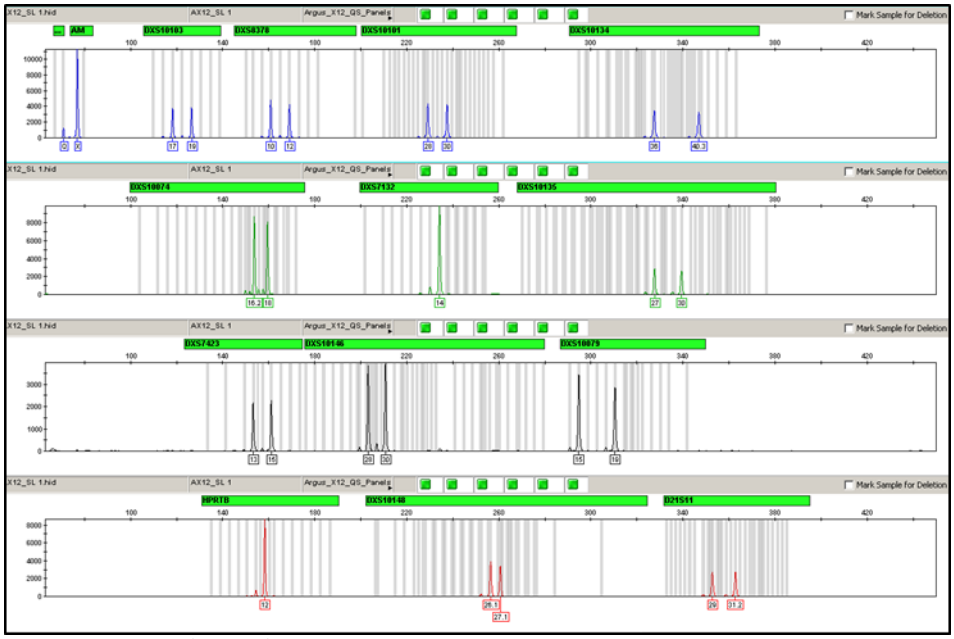


**Figure 24. Direct amplification of buccal swabs.** Buccal swabs were lysed for 5 min at 95°C using the Investigator STR Lysis Buffer. 2 µl lysate was amplified in 28 PCR cycles.

## References

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   http://media.wix.com/ugd/4344b0_cbc27d16dcb64fd88cb36ab2a2a25e4c.pdf.

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## Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| Investigator Argus X-12 QS Kit (25) | Primer mix, Fast Reaction Mix including HotStarTaq *Plus* DNA Polymerase, Control DNA, allelic ladder Argus X-12 QS, DNA size standard (BTO), and RNase-free water | 383223 |
| Investigator Argus X-12 QS Kit (100) | Primer mix, Fast Reaction Mix including HotStarTaq *Plus* DNA Polymerase, Control DNA, allelic ladder Argus X-12 QS, DNA size standard (BTO), and RNase-free water | 383225 |

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