QIAGEN Validation Report

Developmental validation of the Investigator® Argus X-12 Kit

The QIAGEN® Investigator Argus X-12 Kit is intended for molecular biology applications in forensic, human identity, and paternity testing. It is used for multiplex PCR in forensic casework.

The Investigator Argus X-12 Kit is a unique tool for analyzing X-chromosomal markers, which are highly informative for kinship and paternity testing and give heightened discriminatory power in deficiency cases. It is also well suited for gonosomal STR analysis of forensic stains. As an informative supplement to the Investigator Argus Y-12 QS Kit, it delivers a heightened discriminatory power for deficiency cases.

The Investigator Argus X-12 Kit was validated for various reaction conditions and situations that can arise during its storage and transport, as well as during the processing of forensic samples. 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.

Variations in buffer component concentrations are described using the example of $MgCl_2$ concentration (page 2). A range of thermal cyclers were used to demonstrate the robustness of the assay (page 3). Its sensitivity to different cycle numbers (page 4) and to variations in PCR annealing temperature (page 5) were also assessed, as was its sensitivity to serial dilutions (page 6). The stability of the components was validated with regard to repeated freezing and thawing (page 8), long-term storage (page 9), and transport (page 10). Specific issues that can arise during forensic casework were investigated, including cross-reactivity with non-human DNA (page 11) and inhibition due to contaminants introduced during extraction (page 12). The reproducibility of the results was also verified (page 14).

The validation of the Investigator Argus X-12 Kit showed that it yields robust and reproducible results within the normal range of conditions expected in forensic casework, as well as in long-distance transport and long-term storage.

Note: All of the electropherograms shown were generated on an Applied Biosystems® ABI PRISM® 3130 Genetic Analyzer. The standard conditions specified in the *Investigator Argus X-12 Handbook* were used for the electrophoresis.

Results of developmental validation

Reaction conditions

Reaction conditions were established for an optimal performance in terms of sensitivity, specificity, and reproducibility. This required the optimization of all of the buffer components at various concentrations. The final composition of the designated reaction mix is a robust buffer system that tolerates differences in the concentration of individual buffer components without a decrease in overall kit performance.

For example, various concentrations of $MgCl_2$, one of the critical buffer components, were added to a standard reaction with 500 pg XX28 control DNA (Figure 1). The assay yielded robust results within an $MgCl_2$ concentration range of +100% of the optimum.

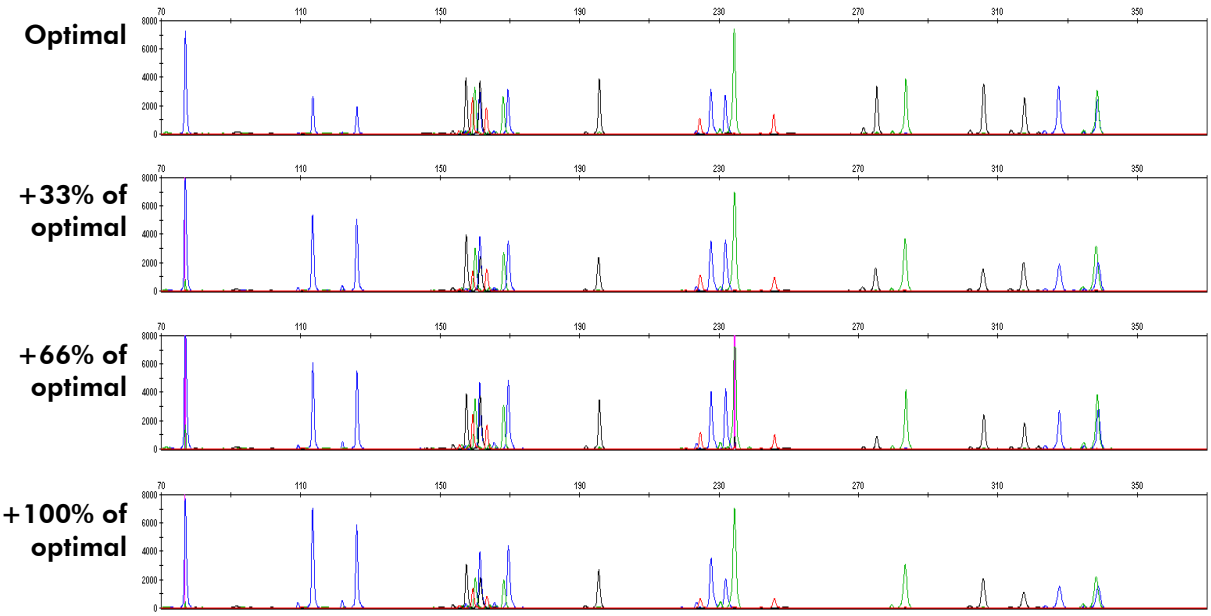

Figure 1. Influence of $MgCl_2$ concentration.

Effect of different cycler types

Multiple PCR thermal cyclers were tested with the Investigator Argus X-12 Kit to demonstrate its robustness. 500 pg Control DNA XX28 was used as a PCR template. The reaction took place under standard conditions and was performed with the following thermal cyclers.

- GeneAmp® PCR System 9700 with Aluminum block (Applied Biosystems Inc., Foster City, CA, USA)
- GeneAmp PCR System 9700 with Silver or Gold-plated Silver block (Applied Biosystems Inc., Foster City, CA, USA)
- DNA Engine® PTC-200 Peltier Thermal Cycler (Bio-Rad Laboratories GmbH, Munich, Germany)
- Techne® TC-512 Thermal Cycler (biostep GmbH, Jahnsdorf, Germany)
- T1 Thermal cycler (Biometra biomedizinische Analytik GmbH, Göttingen, Germany)
- Eppendorf® Mastercycler® ep (Eppendorf AG, Hamburg, Germany)

The electropherograms in Figure 2 show comparable mean peak heights for all of the tested PCR cyclers. No imbalance, dropouts, or preferential amplification for the STR systems was 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".

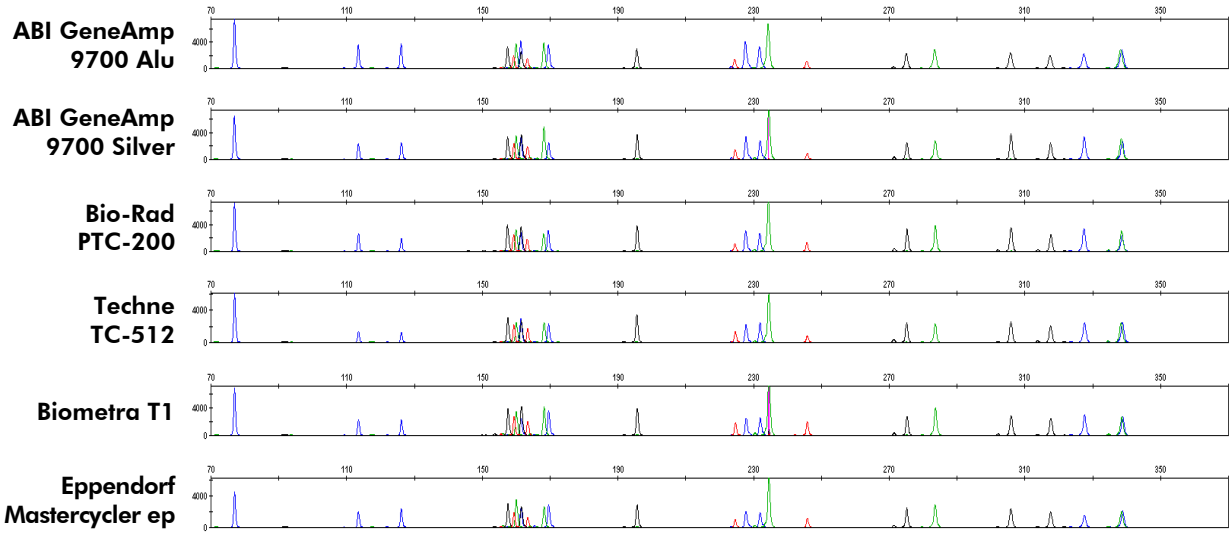

Figure 2. Effect of different cycler types on mean peak height.

Effect of different cycle numbers

Altering the number of cycles can improve amplification when working with low-copy-number DNA.

To test the effect of changing the number of cycles, 500 pg Control DNA XX28 was used for 30 cycles, 200 pg for 32 cycles, and 100 pg for 34 cycles (Figure 3).

Complete profiles were generated under all of the tested conditions. It should be noted that an increase in cycle number to more than 30 may also increase the chance of nonspecific products. Furthermore, because of stochastic effects, peak imbalance or dropouts may be observed for low-copy-number samples (with 100 pg or less of template DNA), regardless of any increase in cycle numbers.

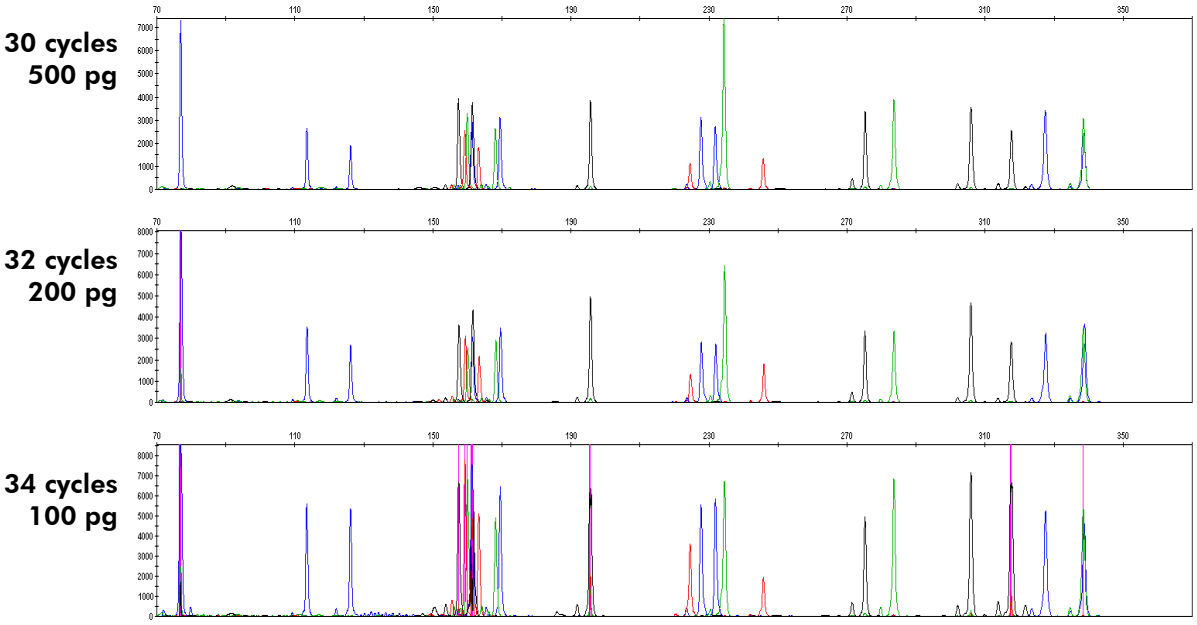

Figure 3. Effect of different cycle numbers on mean peak height. To better visualize smaller peak heights, the y-axis scales were adjusted for best fit.

Effect of PCR annealing temperature variations

Specificity and sensitivity are critical in forensic casework, and both 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 for the Investigator Argus X-12 reaction (63°C for cycles 1 to 5 and 60°C for cycles 6 to 30).

Annealing temperatures of

5 cycles at 61°C, 25 cycles at 58°C

5 cycles at 62°C, 25 cycles at 59°C

5 cycles at 63°C, 25 cycles at 60°C

5 cycles at 64°C, 25 cycles at 61°C

5 cycles at 65°C, 25 cycles at 62°C

were applied to the amplification of 500 pg Control DNA XX28. The PCR was performed on an Eppendorf Mastercycler ep and analyzed on an Applied Biosystems ABI PRISM 3130 Genetic Analyzer.

As shown in Figure 4, no allelic dropout was detected within ±2°C of the optimal T_m of 63 and 61°C. This shows that there is no loss in sensitivity under these conditions. Furthermore, no unspecific peaks occurred, indicating good specificity in this T_m range.

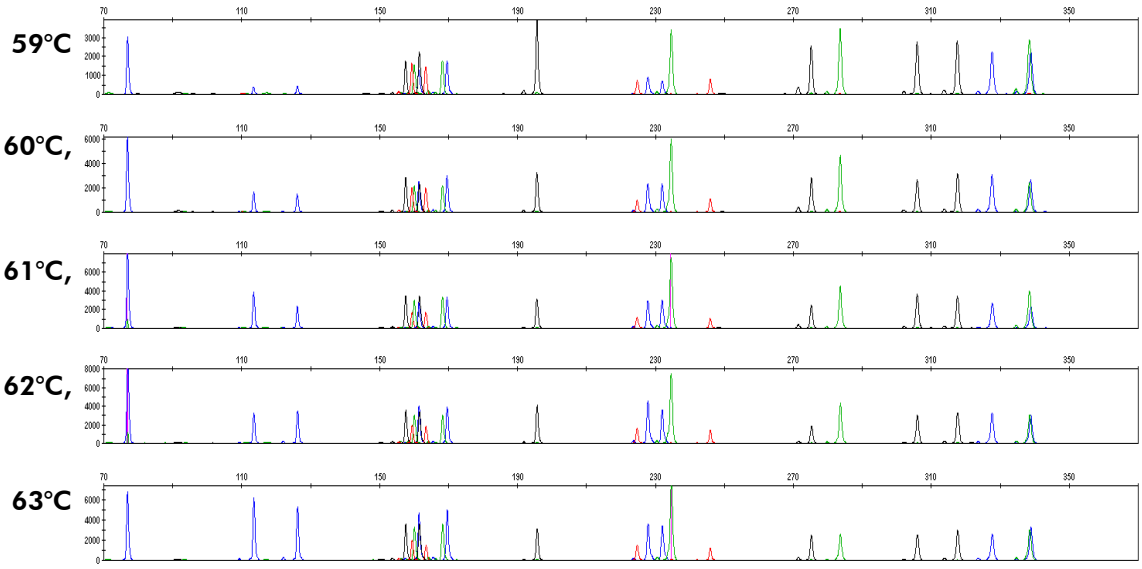

Figure 4. Effect of variations in the PCR annealing temperature. 5x 60°C indicates 5 cycles at 60°C, and so on.

Sensitivity to serial dilutions

The Investigator Argus X-12 Kit is designed to detect a range of DNA quantities. The optimal amount of input DNA to yield good quality STR profiles is 350 pg. Figure 5 (page 7) shows a serial dilution of Control DNA XX28 from 2 ng to 62.5 pg. The optimal linear dynamic range of the assay is in the range of 500 to 125 pg. Full profiles (24 alleles) were consistently obtained at 125 pg using the standard conditions specified in the *Investigator Argus X-12 Handbook*.

Issues with very high amounts of DNA

An amount of DNA above the optimal range (>2 ng) may give inaccurate or unusable data. The fluorescence intensity may cause peaks to go off the scale. Such data cannot be quantified accurately based on peak height and area. As a secondary effect, irregular stutter-peak heights may occur.

"Off scale" peaks are often accompanied by "pull up" peaks. This effect hinders the multi-component analysis, and is caused by an inaccurate spectral separation. 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 DNA.

Issues with very low amounts of DNA

An amount of DNA below the optimal range (<100 pg) may lead to incomplete profiles, where partial profiles lacking alleles occasionally occur. 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 analyzer runs of the same sample to complete the partial profiles, or by adding the maximum volume of the DNA template to the PCR.

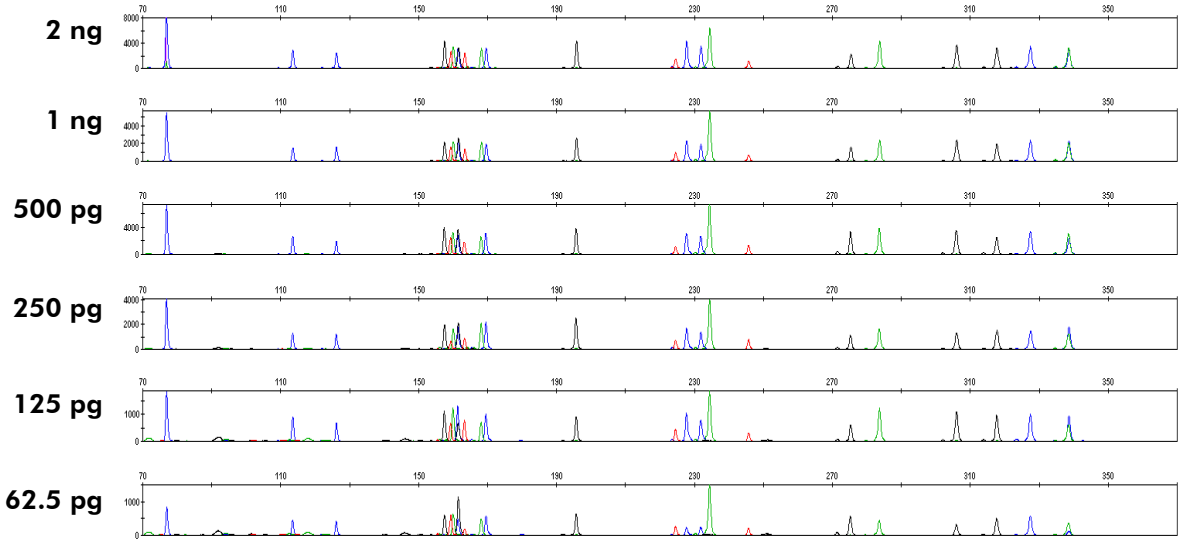

Figure 5. Effect of serial dilution of Control DNA XX28 during amplification. The y-axis scales were adjusted for best fit.

Stability

20 freeze/thaw cycles

In a forensic lab, the maximum number of reactions of a kit may not be performed in a single day. Therefore, the Investigator Argus X-12 components were tested to prove that they would yield stable results after multiple rounds of freezing and thawing. Regardless of these results, we do not recommend repeated freezing and thawing of the kit contents.

Figure 6 shows the electropherograms obtained by amplifying 500 pg Control DNA XX28 with fresh kit components (No Freeze/Thaw) and with kit components stressed by 20 rounds of freezing and thawing (20x Freeze/Thaw). The overall kit performance was not compromised under the chosen conditions. Comparable peak heights were obtained before and after 20 rounds of freezing and thawing.

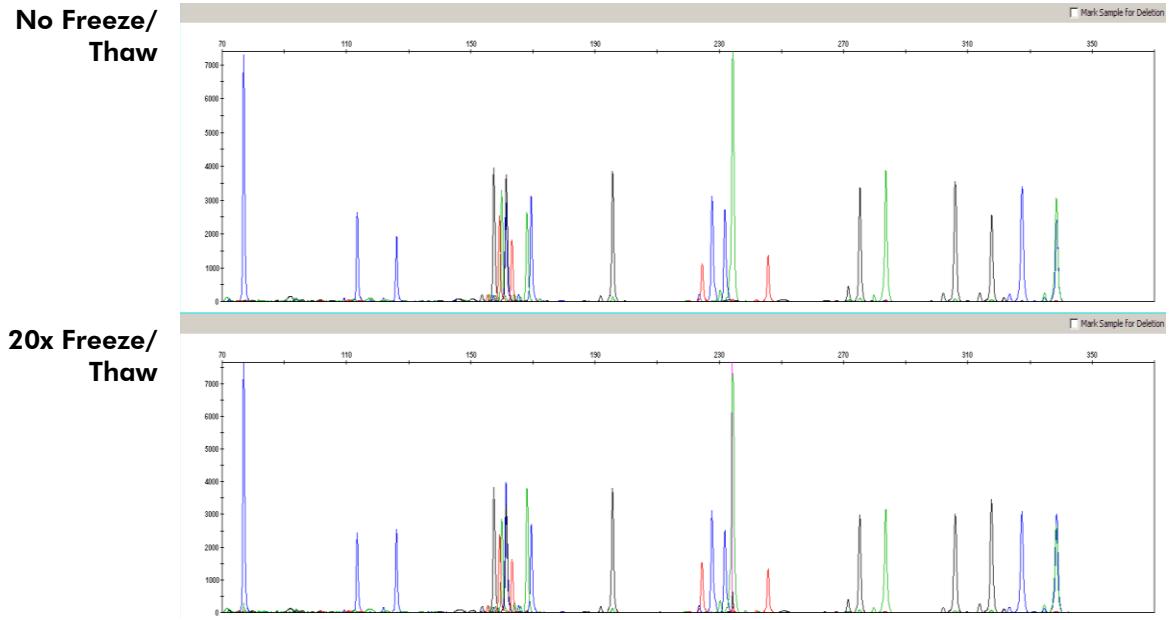

Figure 6. Results of a simulated freeze/thaw stability test of kit components.

Long-term stability

Forensic kits must be viable after long-term storage. Investigator Argus X-12 Kit components were stored for 6 months at –20°C. After 1, 2, 3, 6, 12, 18, and 24 months, the kit performance was tested by amplifying 500 pg Control DNA XX28 under standard PCR conditions. The electrophoresis was performed on an Applied Biosystems ABI PRISM 3130 Genetic Analyzer. Over the course of the experiment, the overall kit performance was found to be stable (Figure 7).

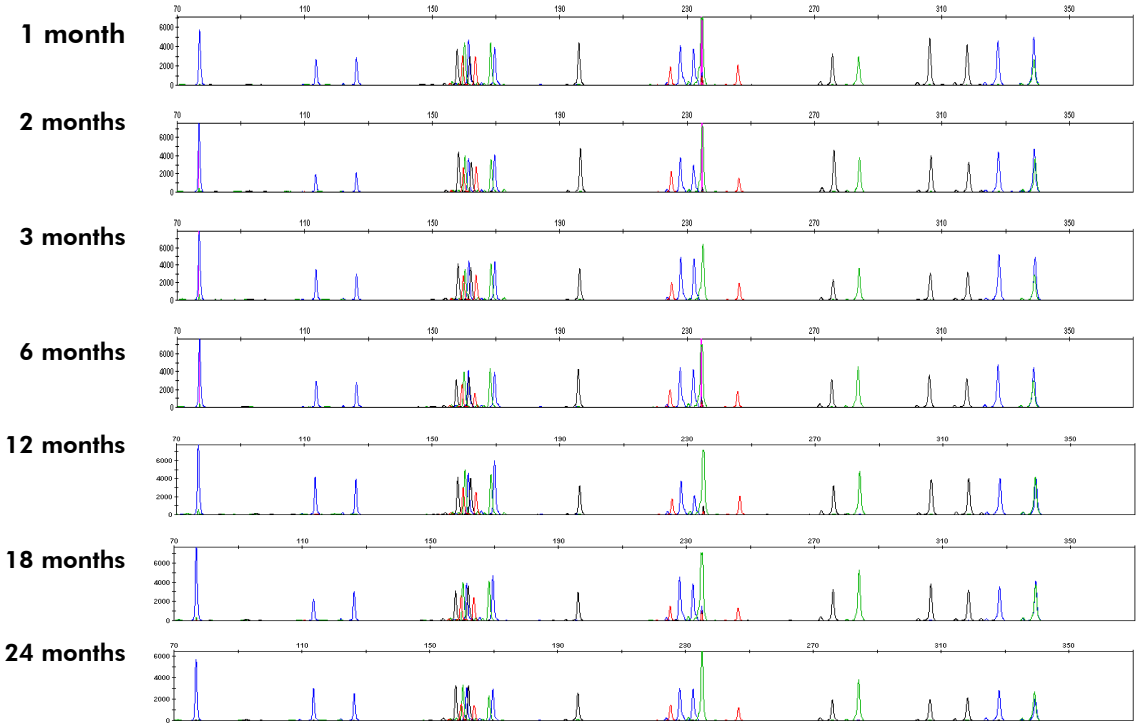

Figure 7. Effects of long-term storage. Kit components were tested at the indicated times.

Simulated shipment condition on dry ice

Investigator Argus X-12 Kits are shipped on dry ice. To assess the performance of the kit after such transportation, the components were stored on dry ice and at –20°C for 5 days.

Kits were stored for 16 h on dry ice, and then transferred to –20°C for 8 h. This cycle was repeated for 5 days. Each day, components from these kits were used to amplify 500 pg Control DNA XX28. The electrophoresis was performed on an Applied Biosystems ABI PRISM 3130 Genetic Analyzer.

The results indicate that the performance before and after storage on dry ice is comparable (Figure 8).

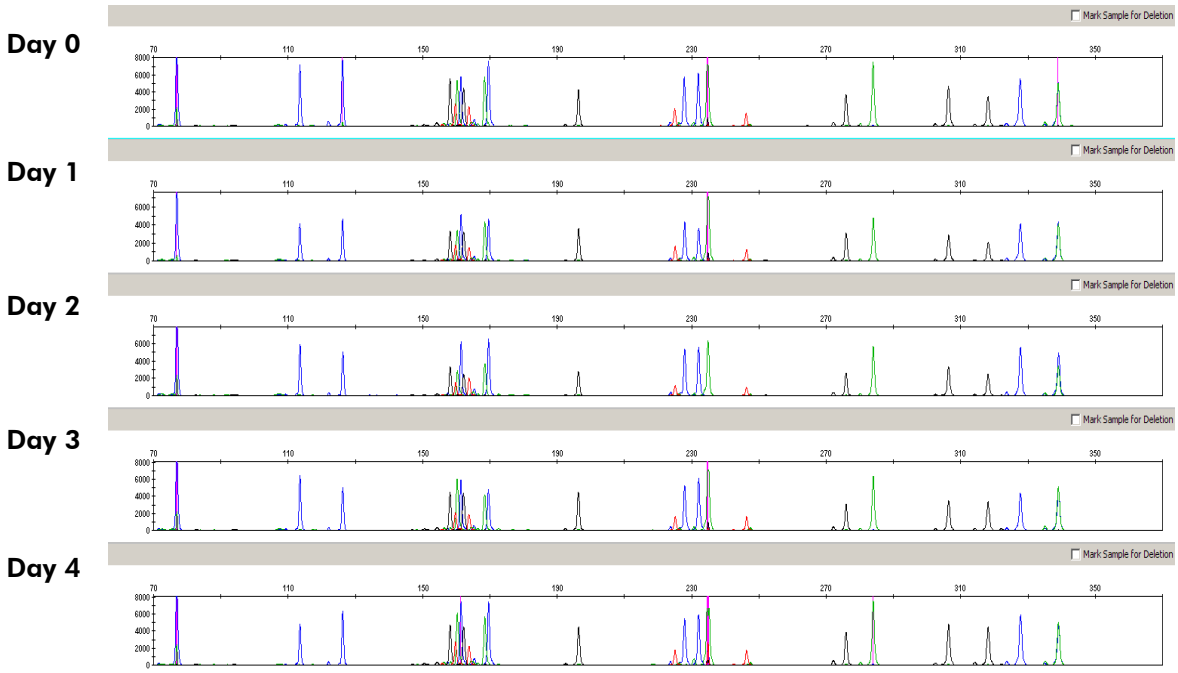

Figure 8. Effect of prolonged storage of Investigator Argus X-12 components on dry ice.

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 species specificity, 2.5 ng of DNA from dog, cat, rabbit, cow, and pig were each tested following the standard assay protocol, with 500 pg Control DNA XX28 as a positive control.

The presence of any amplified peaks in the electropherograms would have suggested cross-reactivity with DNA from the non-human species. None of the tested DNA yielded any detectable product peaks under the chosen conditions, as shown in Figure 9.

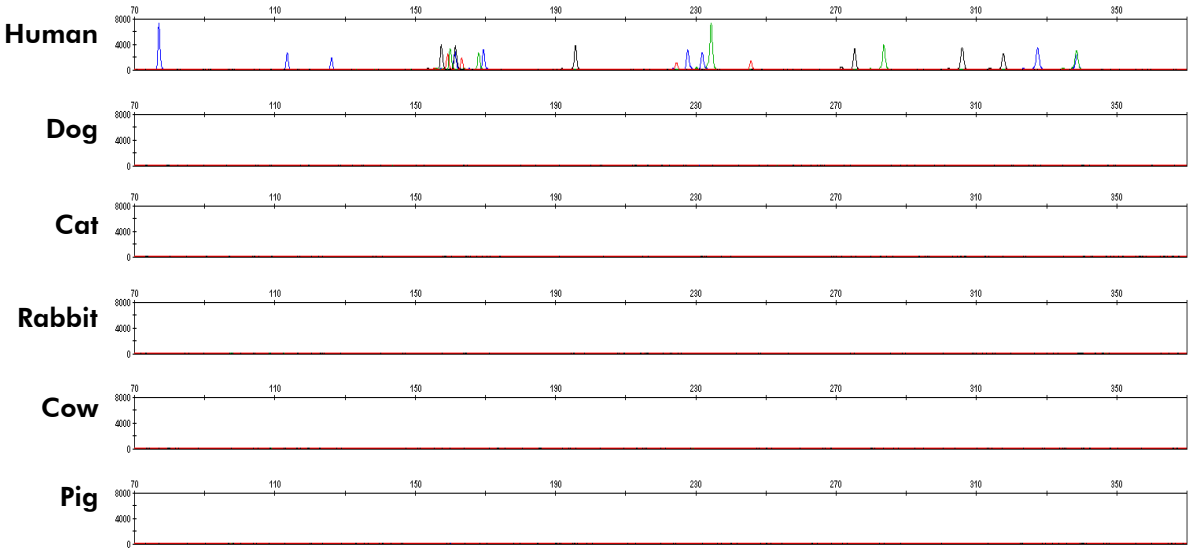

Figure 9. Results of the species specificity assessment.

Performance with simulated inhibition

If the DNA extraction from forensic casework samples is done using inappropriate methods, Investigator Argus X-12 assay performance may be compromised. QIAGEN sample preparation technology is recommended for extraction, as it yields pure DNA, free from inhibitors.

Humic acid, a principal component of humic substances, has an inhibitory effect on PCR. It is often co-purified and co-extracted from forensic samples collected from soil.

To test the robustness of the kit in the presence of typical inhibitors, the assay was run in the presence of 0, 10, 20, 30, 40, and 50 ng/µl humic acid under standard conditions (500 pg Control DNA XX28, 30 cycles).

Hematin is 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. Its interfering effect is related to the inhibition of polymerase activity.

Investigator Argus X-12 Kit performance was assessed in the presence of increasing concentrations of hematin: 0, 50, 100, 150, 180, and 200 µM under standard conditions (500 pg Control DNA XX28, 30 cycles).

Full profiles were obtained in the presence of 40 ng/µl humic acid and 150 µM hematin. The results for these maximum inhibitor concentrations from both studies are shown in Figure 10 (page 13).

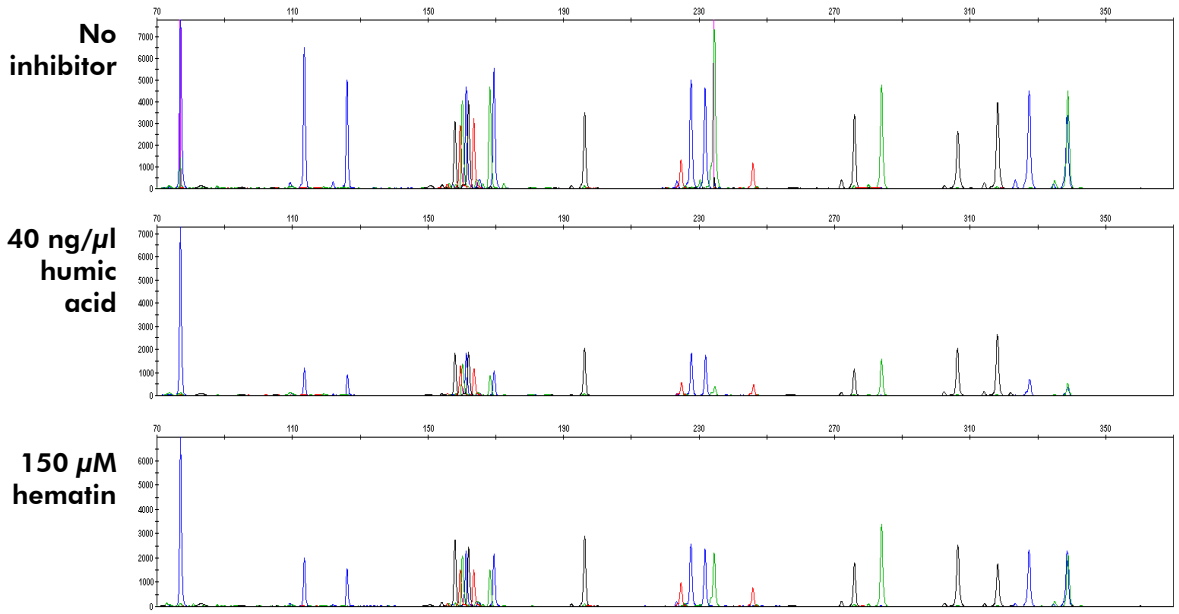

Figure 10. Impact of humic acid and hematin on performance. The y-axis scales were adjusted for best fit.

Reproducibility

Test with an internal DNA pool

The reproducibility of results generated by the Investigator Argus X-12 was validated under standard conditions. This included a test of automatic allele calling based on the allelic ladder, and a concordance analysis of the allele correlation compared to Control DNA that had been pre-validated in an additional forensic assay.

This assay partially covered the STR systems of interest. The kit used was the Mentype® Argus X-8 (Biotype Diagnostics GmbH, Dresden, Germany).

A total of 80 DNA samples (40 male, 40 female) of different origin (blood, saliva) were quantified, and profiles were generated with the Investigator Argus X-12 in quadruplicate. The assay produced full profiles with steady peak heights of >50 rfu. The profiles generated with Investigator Argus X-12 kits concurred with those generated by the Mentype Argus X-8 assay.

Ordering Information

Product	Contents	Cat. no.
Investigator Argus X-12 Kit (25)	Primer mix, reaction Mix, Multi Taq2 Polymerase, Control DNA, DNA Size Standard 550 (BTO), allelic ladder Argus X-12, and nuclease-free water	383213
Investigator Argus X-12 Kit (100)	Primer mix, reaction Mix, Multi Taq2 Polymerase, Control DNA, DNA Size Standard 550 (BTO), allelic ladder Argus X-12, and nuclease-free water	383215

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Service or your local distributor.

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