Improving STR analysis using a novel quantification technology — more than simply DNA quantification

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Introduction

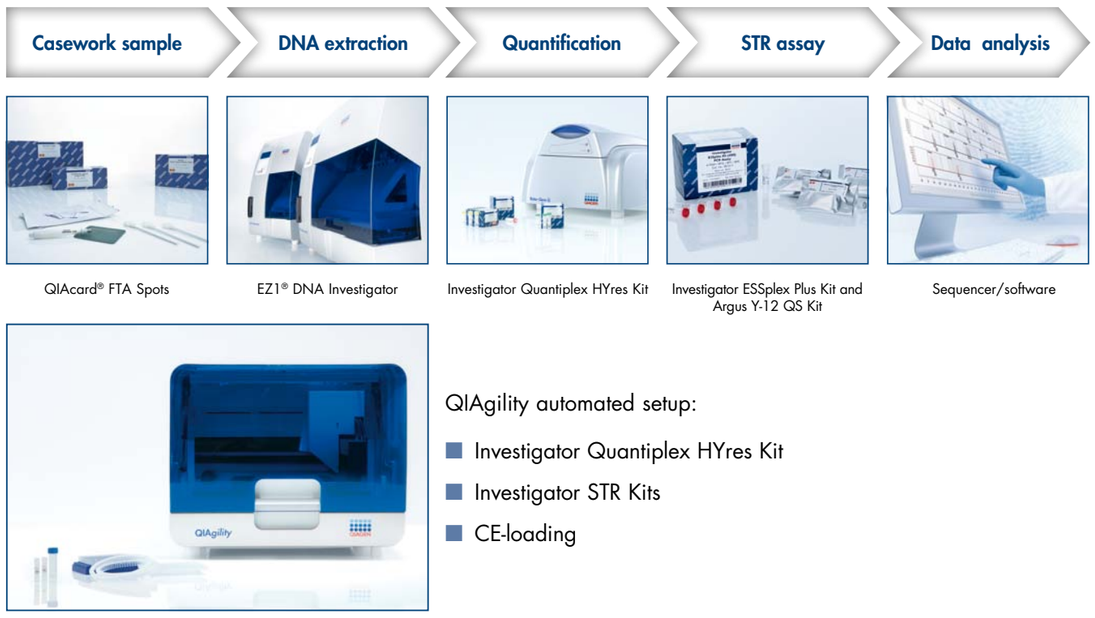
Commonly, short tandem repeat (STR) analysis is performed for human identification (HID), although alternative approaches such as the analysis of deletions insertions polymorphisms (DIPs) are now available. However, these multiplex assays used for HID are complex and require a defined range of template input. Accurate quantification (even at low concentration) and assessment of the presence of PCR inhibitors are key to ensuring successful genotyping at the first attempt.

Quantification in forensic casework analysis, mostly performed using real-time PCR techniques, is typically the only pre-STR analysis step and should be seen as a quality control step rather than just a quantification of the DNA concentration. Therefore, there is a need for advanced solutions that further improve the forensic workflow and increase the accuracy by reducing the time to results.

The novel Investigator® Quantiplex HYres Kit provides fast and accurate quantification of total human and male DNA in forensic database and casework samples. The assay provides sensitivity down to 0.3 pg/µl for both targets, with highly accurate quantification in linear range of the standard curve down to 4.9 pg/µl for the human and 6.5 pg/µl for the male target. A balanced internal amplification control ensures detection of PCR inhibitors. Both the Investigator Quantiplex and the Investigator Quantiplex HYres assays use PCR fast-cycling technology for rapid results. When used with the Rotor-Gene® Q system, quantification is achieved in about 50 minutes. To further streamline the workflow and to minimize time-consuming and error-prone manual steps, it is possible to combine both kits with the QIAsymphony® or QIAgility® instruments allowing automation of routine procedures in the forensic laboratory workflow. Combining the assay with instrumentation significantly shortens time to result, with increased accuracy and sensitivity.

Materials and methods

The Investigator Quantiplex HYres Kit was used for real-time PCR quantification of samples. All steps were automated using the QIAgility, including reaction setup for real-time PCR (including CE setup). A swab of a plastic bottle was used for simulation of a casework sample.



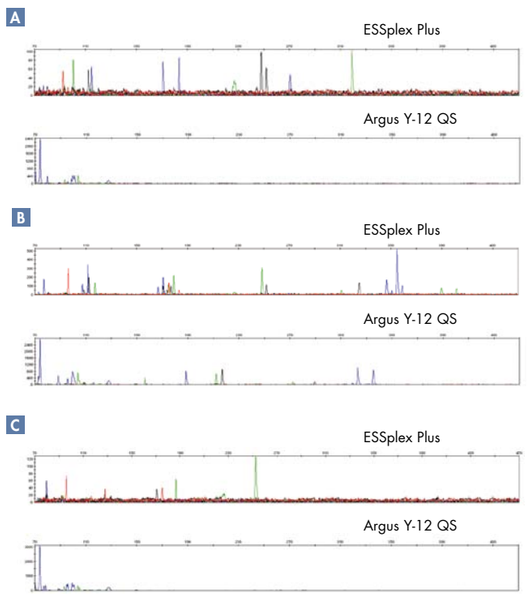
Results: Reliable quantification down to <1 pg/µl DNA

The Investigator Quantiplex HYres Kit was used for quantification of a simulated casework sample on the Rotor-Gene Q. The DNA was isolated using a swab to collect DNA samples from a prepared plastic bottle which was touched by a single male and a single female. The DNA was isolated using the EZ1 DNA Investigator Kit and analyzed in duplicate. The table shows the results of the quantification and of the STR reaction using both the Investigator ESSplex Plus Kit and the Investigator Argus Y-12 QS Kit. The quantification yielded expected results for this kind of mixed sample with low DNA concentrations in the stochastic range (all below the standard curve range of Quantiplex HYres). Nevertheless, we demonstrated that the quantification results of Quantiplex HYres correlate to the STR results both for the autosomal and Y-STR analysis.

Sample 1 showed for both the human and the male target DNA concentration below 1 pg/µl, and the STR showed sporadic alleles. Sample 2 showed higher DNA concentrations for both the human and the male target and partial profiles were detected. Sample 3 showed no DNA for both the human and the male target and no profiles were detected.

	Quantification			ESSplex Plus		Argus Y-12 QS	
Sample	**Measured concentration human DNA (pg/µl)**	**Measured concentration male DNA (pg/µl)**	**Replicates**	**DNA amount in STR (using 15 µl DNA)**	**Results (2 replicates)**	**DNA amount in STR (using 16.9 µl DNA)**	**Results (1 replicate)**
Sample 1	0.79 ± 0.07	0.17 ± 0.14	2/2	11.8 pg	10/116	2.8 pg	0/12
Sample 2	1.39	0.54	1/2	20.8 pg	45/116	8.1 pg	10/12
Sample 3	0	0	0/2	0 pg	3/116	0 pg	0/12

Results: Example of improved correlation with STR results



DNA profiling of a simulated touch sample was performed using both the Investigator ESSplex Plus Kit and the Investigator Argus Y-12 QS Kit. The threshold used for all experiments was 100 RFU. The correlation between DNA quantification and STR profile is shown.

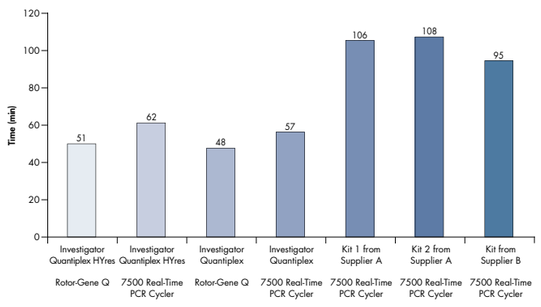
- STR of Sample 1 where very low DNA amounts were detected using the Investigator Quantiplex HYres Kit shows sporadic alleles.
- STR of Sample 2 where higher DNA amounts were detected using the Investigator Quantiplex HYres Kit shows partial profiles.
- STR of Sample 3 where no DNA was detected using the Investigator Quantiplex HYres Kit shows no useful profiles.

The quality sensor QS in the Argus Y-12 Kit performed as expected for all samples.

DNA profiling of simulated casework samples using the Investigator ESSplex Plus Kit or Investigator Argus Y-12 QS Kit. STR profiles obtained from a DNA sample isolated from the surface of a plastic bottle using the EZ1 DNA Investigator Kit. 15 µl DNA was used for autosomal DNA profiling. 16.9 µl DNA was used for Y-STR profiling. **A** STR profiles from Sample 1. Measured DNA concentrations: Human: 0.79 pg/µl; male: 0.17 pg/µl **B** STR profiles from Sample 2. Measured DNA concentrations: Human: 1.39 pg/µl; male: 0.54 pg/µl. **C** STR profiles from Sample 3. Measured DNA concentrations: Human: 0 pg/µl; male: 0 pg/µl. PCR was performed using the GeneAmp® PCR System 9700 using 30 cycles. 1 µl of each PCR product was added to 12 µl Hi-Di™ Formamide/Size Standard mix. Capillary electrophoresis was performed on an Applied Biosystems® 3500 Genetic Analyzer for Human Identification.

Results: Shorten time to results using fast PCR cycling protocol with novel PCR chemistry and Scorpion primers

The Investigator Quantiplex HYres Kit provides highly accurate results in 51 minutes using the Rotor-Gene Q. It is also compatible with other instruments, although lengths of protocols are variable due to the slower heating and cooling rates of conventional block cyclers, compared with the rapid Rotor-Gene Q. The time savings using Quantiplex HYres on the Rotor-Gene Q may amount to up to 1 hour.



Comparison of lengths of cycling protocol on different real-time PCR instruments.

The Rotor-Gene Q.

- Highly accurate and sensitive results in 51 minutes using the Rotor-Gene Q
- Compatible with conventional block cyclers

Conclusions

- The new Investigator Quantiplex HYres Kit significantly shortens time to results in forensic DNA quantification with increased accuracy and sensitivity.
- Through novel PCR-reaction technology, quantification results can be obtained in 51 minutes using the Rotor-Gene Q.
- Highly reliable and accurate quantification down to 4.9 pg/µl or less.
- The high correlation between DNA quantification and STR profile were shown using simulated casework samples.
- All steps were automated using both the QIAgility and the QIAsymphony, including reaction setup of real-time PCR (including samples, standards and controls, normalization of sample DNA concentration, setup of the STR reaction, and CE).

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 Services or your local distributor.

The Investigator ESSplex Plus and Argus Y-12 QS Kits are not available in Canada or the USA.

The QIAgility.

The QIAsymphony RGQ.

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