

Investigator® Quantiplex Kit: For reliable quantification of human DNA in forensic samples



Francesca Di Pasquale, Stefan Cornelius, Margaretha König, Mario Scherer, Claudia Schmid, Claudia Dienemann, Lars Bochmann, Anke Prochnow, Thomas Schnibbe, and Holger Engel

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

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.

Quantitative real-time PCR is the standard method for quantification of DNA in forensic samples. However, there is a need for advanced solutions that further streamline the forensic workflow by increasing the accuracy of and reducing the time to results. The Investigator Quantiplex Kit provides fast and accurate quantification of human DNA in forensic database and casework samples. The assay provides sensitivity down to 0.3 pg/μl, with highly accurate quantification in linear range of standard curve of 4.9 pg/μl. A balanced internal amplification control ensures detection of PCR inhibitors. The Investigator Quantiplex assay uses PCR fast-cycling technology for rapid results. When used with the Rotor-Gene® Q real-time PCR system, quantification is achieved in 48 minutes. To further streamline the workflow and to minimize time-consuming and error-prone manual steps, it is possible to combine the Investigator Quantiplex Kit with the QIAgility® instrument, a bench-top instrument allowing automation of routine procedures in the forensic laboratory workflow, such as quantification and STR setup. Combining this innovative real-time PCR-based quantification tool with liquid-handling instrumentation significantly shortens hands-on time and overall time to result, with increased accuracy and sensitivity. In addition, overall process safety is enhanced.

Materials and methods

The Investigator Quantiplex 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 cigarette butt was used for simulation of a casework sample.



- QIAgility automated setup:
- Investigator Quantiplex Kit
 - Investigator ESSplex Kit
 - CE-loading

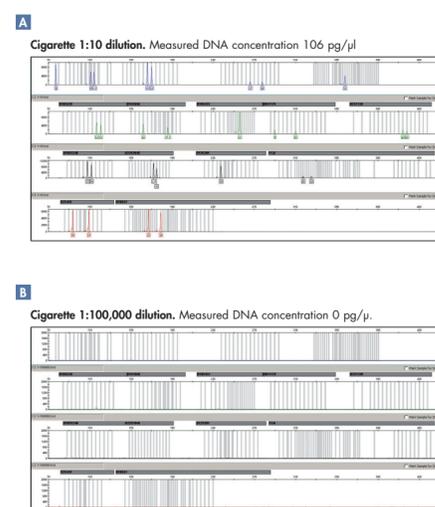
Results: Quantification using Investigator Quantiplex Kit

The Investigator Quantiplex Kit was used for quantification of a simulated casework sample on the Rotor-Gene Q. The DNA was isolated from a cigarette butt using the EZ1 DNA Investigator Kit. Duplicates of a DNA dilution series were analyzed. The table shows the results of the quantification and of the STR reaction using the Investigator ESSplex Kit. The quantification yielded expected results for the undiluted sample and dilutions between 1:10 and 1:1000, with an STR profile (full or partial) detected for all samples. The 10,000 fold dilution is the detection limit of the quantification reaction and the STR reaction showed sporadic alleles. The 100,000 fold dilution could not be quantified and no STR profile was detected.

Sample	Quantification results		ESSplex results	
	Measured concentration (pg/μl)	Replicates	DNA amount in STR (using 10 μl DNA)	ESSplex results (2 replicates)
Cigarette (undiluted)	1012	2/2	N/A	N/A
Cigarette 1:10	106	2/2	1060 pg	56/56 (full profile)
Cigarette 1:100	9.7	2/2	97 pg	56/56 (full profile)
Cigarette 1:1,000	0.3	2/2	3 pg	22/56 (partial profile)
Cigarette 1:10,000	0.3*	1/2*	3 pg*	5/56 (sporadic alleles)
Cigarette 1:100,000	0	0/2	0	0/56 (no profile)
NTC	0	0/2	0	0/56 (no profile)

* Only 1 replicate out of 2 showed a result; stochastic effects may be expected at concentrations 2 pg/rxn.
N/A: Not applicable; NTC: No-template control.

Results: Improved correlation of quantification with STR results



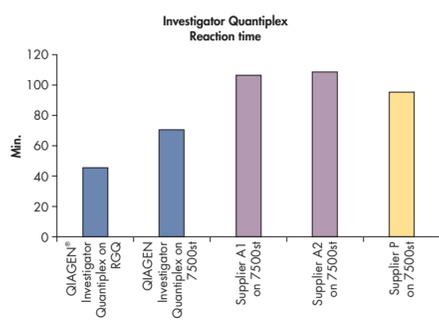
The DNA profiling of a simulated casework sample was performed using the Investigator ESSplex Kit. The correlation between DNA quantification and STR profile is shown.

- STR of the 10-fold dilution where the DNA amount was detected using the Investigator Quantiplex Kit shows a full profile
- STR of the 100,000-fold dilution where no DNA was detected using the Investigator Quantiplex Kit shows no profile

DNA profiling of simulated casework samples using the Investigator ESSplex Kit. Reaction setup and CE-loading were performed using the QIAgility. **A** STR profile obtained from a 10-fold dilution of a DNA sample extracted from a cigarette butt using the EZ1 DNA Investigator Kit. 10 μl DNA was used for DNA profiling. **B** STR profile obtained from a 100,000-fold dilution of a DNA sample extracted from a cigarette butt using the EZ1 DNA Investigator Kit. 10 μl DNA was used for DNA profiling. PCR was performed on ABI GeneAmp® 9700 using 30 cycles. 1 μl of each PCR product was added to 12 μl Hi-Di™ Formamide/Size Standard mix on the QIAgility.

Results: Fast PCR cycling protocol with novel PCR chemistry and Scorpion primers

The Investigator Quantiplex Kit provides highly accurate results in 48 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.



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

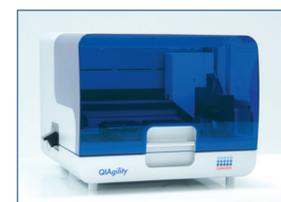


The Rotor-Gene Q.

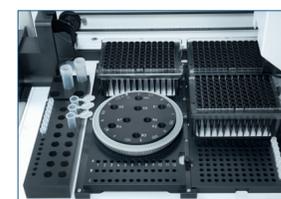
- Highly accurate and sensitive results in 48 minutes using the Rotor-Gene Q
- Compatible with conventional block cyclers.

Conclusions

- The new Investigator Quantiplex Kit shows increased accuracy and sensitivity down to 0.3 pg/μl.
- The novel PCR reaction technology enables quantification results in 48 minutes using the Rotor-Gene Q.
- The high correlation between DNA quantification and STR profile was shown using simulated casework samples.
- All steps were automated using the QIAgility, including reaction setup of real-time PCR (including samples, standards and controls, normalization of sample DNA concentration, setup of the STR reaction and CE).



The QIAgility.



The QIAgility worktable.

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The Investigator ESSplex Kit is not available in Australia, Canada, or the USA.

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