

Streamlined forensic workflow with the Investigator™ Quantiplex Kit and automated reaction setup



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.

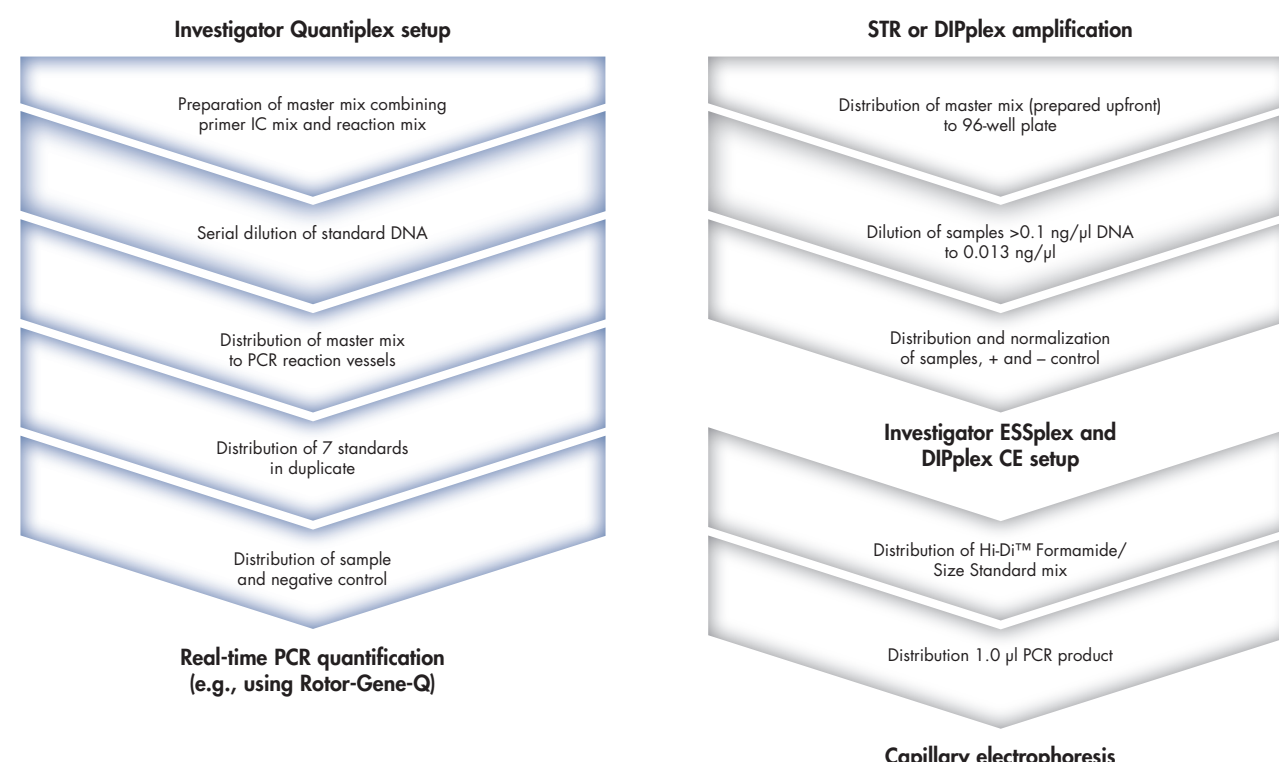
Quantitative real-time PCR is the standard method for quantification of DNA in forensic samples. However, there is a need for advanced solutions that further streamline the forensic workflow by increasing the accuracy of and reducing the time to results. The Investigator Quantiplex Kit provides fast and accurate quantification of human DNA in forensic database and casework samples. The assay provides sensitivity down to <2 pg/reaction, with highly accurate quantification in linear range of standard curve of <10 pg/reaction. A balanced internal amplification control ensures detection of PCR inhibitors. The Investigator Quantiplex assay makes use of PCR fast cycling technology allowing rapid results. When used with the Rotor-Gene® Q system, quantification is achieved in under 50 minutes.

Automation of laboratory procedures is gaining more and more importance in the forensic laboratories, saving time for routine procedures and avoiding user errors.

The QIAgility® system is a bench-top instrument allowing automation of routine procedures in the forensic laboratory workflow, involving PCR setup for real-time PCR-based DNA quantification. The instrument also allows automated adjustment of DNA concentration in forensic samples to a specified concentration, making use of real-time PCR-based quantification results. The reaction setup for the Quantiplex HID assay can be performed by the instrument. Combining the assay with instrumentation significantly shortens time to result, with increased accuracy and sensitivity. In combination with the QIAgility, the workflow can be further streamlined and time-consuming, error-prone manual interactions are minimized.

Materials and methods

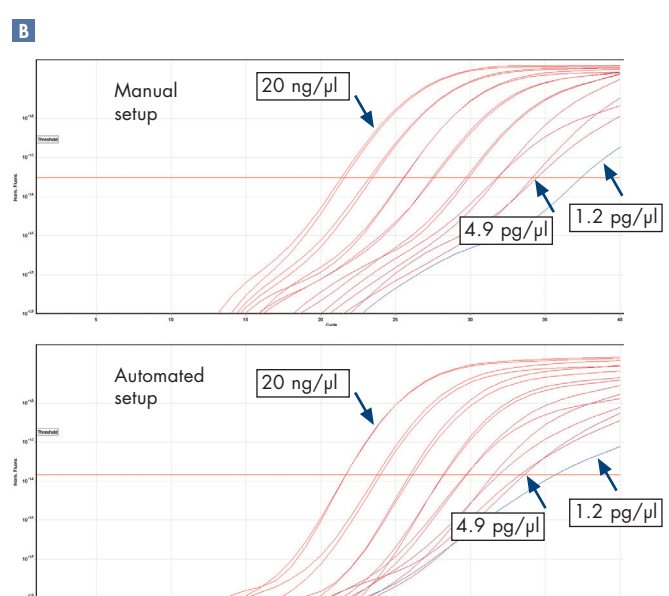
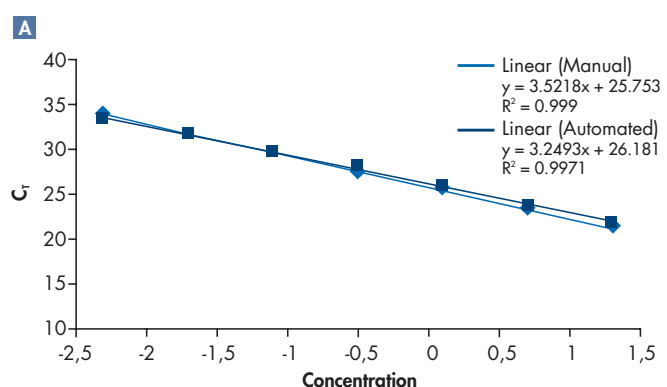
The Investigator Quantiplex Kit was used for real-time PCR quantification of samples. All steps were automated using the QIAgility, including reaction setup for real-time PCR (including CE setup). The DNAs used were the test DNAs XYa (male) and XXb (female).



Results: Investigator Quantiplex PCR Kit

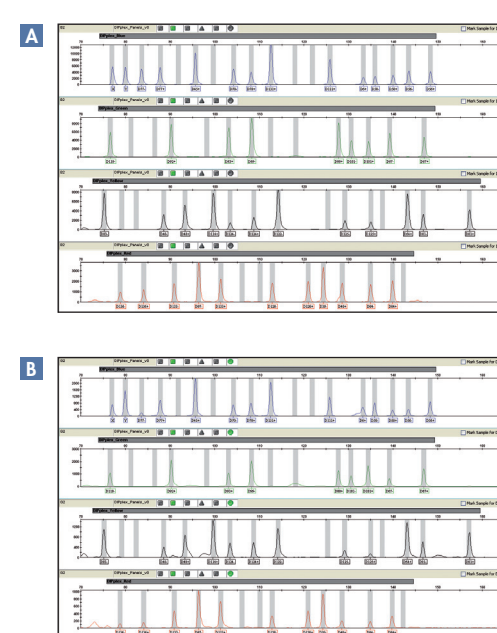
The Investigator Quantiplex Kit PCR Kit was used for quantification of 56 human DNA samples on the Rotor-Gene Q. Manual and automated setup were compared. Duplicates of a dilution series of the Control DNA Z1 and of two different test DNAs (XYa and XXb) were analyzed. The PCR setup was performed manually and automated in parallel using the QIAgility.

- Highly linear standard curve down to 4.9 pg/µl.
- High accuracy at low concentrations.
- Consistent results of manual and automated reaction setup.



A Standard curve using the Investigator Quantiplex Kit. Control DNA Z1 was consecutively diluted by a factor of 4 either manually or automated using the QIAgility. The resulting standard series were amplified in duplicates. Reaction efficiency and R² value are shown.
B Amplification curves of the Investigator Quantiplex Kit on the Rotor-Gene Q, with reaction setup performed manually or using the QIAgility. Reproducible results are obtained down to 4.9 pg/µl (red curves; C_v: 0.59%), with detection of as little as 1.2 pg/µl (blue curve).

Results: Use of concentration-normalized samples in Investigator PCR assay reactions



A Samples of different DNA concentrations were normalized on the QIAgility and used for Investigator DIPplex reaction setup. **B** Control DNA XYa (0.16 ng/µl) was diluted to 0.013 ng/µl and 1.5 µl used as template in the DIPplex reaction. **C** 1.5 µl Control DNA XYa (4.9 pg/µl) was used undiluted as template in the DIPplex reaction. PCR was performed on an 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.

The protocol allows preparation of DIPplex reactions for up to 96 samples, including positive and negative controls, per run.

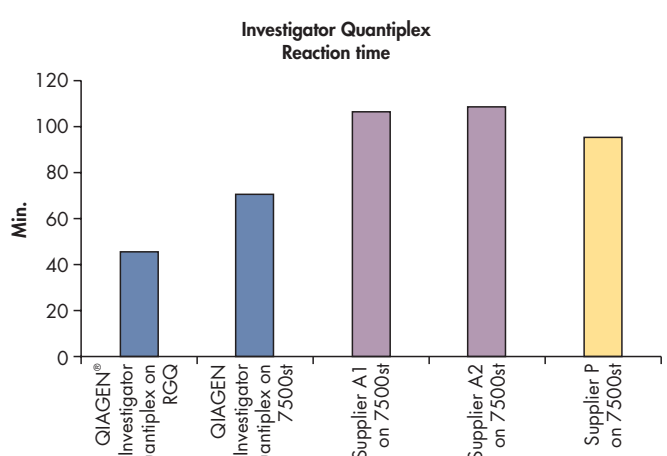
Sample DNAs are normalized to 0.2 ng per reaction based on DNA concentration values imported into the QIAgility software. Samples with concentrations of >0.1 ng/µl (up to 8 ng/µl) are automatically diluted to 0.013 ng/µl and 1.5 µl of the diluted eluate is added to each reaction. Samples whose concentrations are too low to allow the input of 0.2 ng DNA into the assay are added at the maximum possible volume.

After amplification on an ABI GeneAmp® 9700 instrument, samples can be prepared for capillary electrophoresis by adding Hi-Di Formamide/Size Standard mix on the QIAgility.

- Normalization of samples results in uniform peak heights over a broad range of starting concentrations.
- Compatible with DIPplex and STR (data not shown for STR setup).

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

The Investigator Quantiplex Kit provides highly accurate results in under 50 minutes using the Rotor-Gene Q. It is also compatible with other instruments, although lengths of protocols is limited 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.

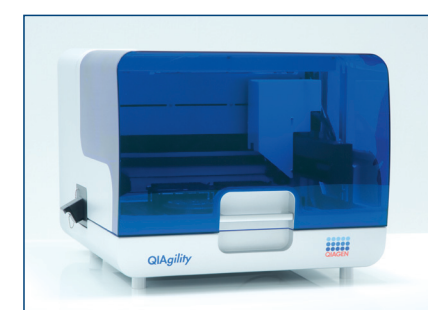
The Rotor-Gene Q.

- Highly accurate and sensitive results in less than 50 minutes using the Rotor-Gene Q.
- Compatible with conventional block cyclers.

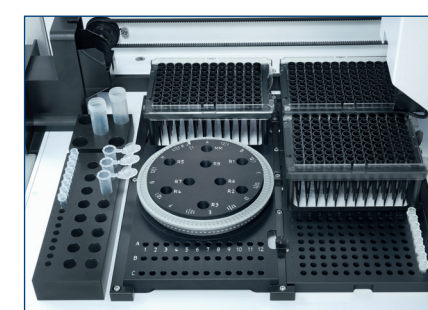
Conclusions

- The new Investigator Quantiplex 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 under 50 minutes using the Rotor-Gene Q.
- Highly reliable quantification down to 5 pg/µl or less.
- 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 or DIPplex reaction and CE).
- Combination of Investigator Quantiplex Kit and automation using the QIAgility further streamlines the forensic workflow, for fast, accurate, and sensitive real-time PCR quantification and elimination of error-prone manual interactions.

QIAGEN products presented here are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.



The QIAgility.



The QIAgility worktable.

Trademarks: QIAGEN®, QIAgility®, Investigator™ (QIAGEN Group); Hi-Di™, GeneAmp® (Life Technologies Corporation); Rotor-Gene® (Corbett Research). © 2010 QIAGEN, all rights reserved.