Development of a new Robust qPCR-based Quantification Assay for Examining Quality and Integrity of Human DNA in Forensic Samples

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Introduction

STR analyses are complex multiplex systems that require a defined range of input DNA and template quality to perform accurately. Therefore, human DNA – which can be isolated from a variety of sources – must be assessed in terms of quantity, quality and integrity before STR analysis. It is essential to extract as much information as possible from the DNA quantification reaction to aid the setup of STR reactions.

The new Investigator® Quantiplex Pro enables the determination of the amount of amplifiable DNA, presence of inhibitors and integrity of the DNA sample in one reaction, ensuring a high correlation between quantification and STR results.

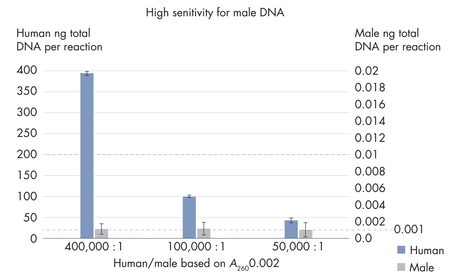
The qPCR assay uses a novel PCR fast-cycling technology and provides rapid, robust and precise quantification and a high sensitivity for male DNA, even in the presence of high amounts of female DNA. The Investigator Quantiplex Pro Kit provides fast and accurate quantification of human DNA in forensic samples in less than 1 hour, with sensitivity down to 0.125 pg/µl and highly accurate quantification in the linear range – down to 0.5 pg/µl. A balanced internal amplification control ensures reliable detection of PCR inhibitors. A novel system for DNA degradation detection allows for precise assessment of the degradation status of the DNA.

To minimize time-consuming and error-prone manual steps, the Investigator Quantiplex Pro Kit can be combined with the QIAgility® or QIAsymphony®. Automation allows for streamlining of routine procedures in the forensic laboratory workflow, such as quantification and STR setup.

High Sensitivity for Male DNA

The Investigator Quantiplex Pro Kit provides high sensitivity and detects low amounts of male DNA, even in a very high background of female DNA (up to 400,000:1).

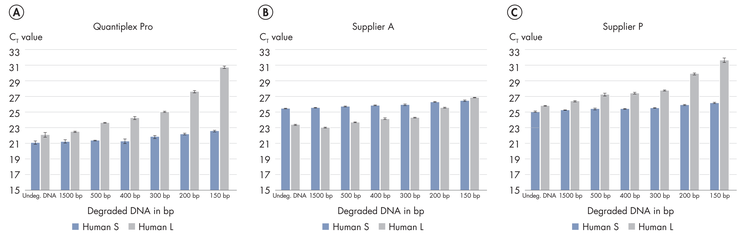
The three tested mixture sets included a constant amount of male DNA (1 pg in total) while the amount of female DNA was increased from 50 ng to 400 ng in total. The Investigator Quantiplex Pro Kit accurately quantified the constant amount of male DNA (at ~1 pg) and the increasing amount of female DNA.



Highly accurate and sensitive results for male DNA. Three different mixture sets were tested in quadruplicate. Each set contains a total of 1 pg of male DNA, while the amount of female DNA was increased from 50 ng to 400 ng.

Development of a System to Detect DNA Degradation

Environmental degradation may occur with forensic casework samples and is a classic challenge in routine genetic fingerprinting. Data presented for the Investigator Quantiplex Pro Kit demonstrate the performance of the newly developed system for accurate detection of DNA degradation.

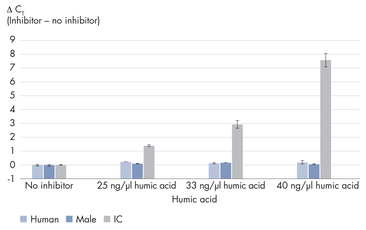


Superior detection of DNA degradation with the Investigator Quantiplex Pro Kit. Male genomic DNA was sheared with a Covaris® S220 Focused-ultrasonicator™ to average fragment sizes of 1500 bp, 500 bp, 400 bp, 300 bp, 200 bp and 150 bp. Ⓐ Each fragment size (4.6 ng) was tested with the newly developed Quantiplex Pro degradation detection system. The performance of two other commercially available kits under the same test conditions is shown in Ⓑ and Ⓒ. All reactions were setup and run according to the manufacturer's instructions.

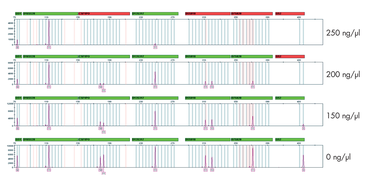
The newly developed system for DNA degradation allows for a more precise assessment of the degradation status of the DNA compared to other kits from other suppliers.

- C_T values for the newly developed large autosomal marker increase consistently with decreasing DNA fragment length
- A maximal difference of eight C_T values difference is reached at 150 bp

Development of A Reliable Internal Control



Performance of the Investigator Quantiplex Pro Kit with simulated humic acid inhibition on the Applied Biosystems 7500 Real-Time PCR System for Human Identification. The internal control reports the presence of the inhibitor (ΔC_T) while quantification is reliable up to a 40 ng/µl. Average ΔC_T ±standard deviation.



Humic acid has an inhibitory effect on PCR and is often co-purified from forensic samples collected from soil.

To test the robustness of the Investigator Quantiplex Pro Kit, the assay was run in the presence of humic acid. The internal control (IC) acts as quality sensor and reports the presence of the inhibitor with a C_T shift, while quantification remains reliable up to a final concentration of 40 ng/µl. This corresponds to a concentration in the STR reaction of 250 ng/µl, if the maximum template volume (15 µl) is applied.

The Investigator 24plex QS Kit shows resistance to humic acid of up to 200 ng/µl (final concentration in the reaction), while the quality sensor drops out and reports inhibitor presence.

- The IC is more sensitive toward inhibitors; quantification results remain reliable
- The response of IC to inhibitors correlates to STR
- IC of Quantiplex Pro reflects the QS of the STR profile

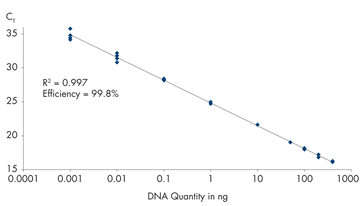
The Investigator 24plex QS Kit shows resistance to humic acid of 500 pg DNA up to 200 ng/µl; a full DNA profile can be still obtained.

Exceptionally High Dynamic Range

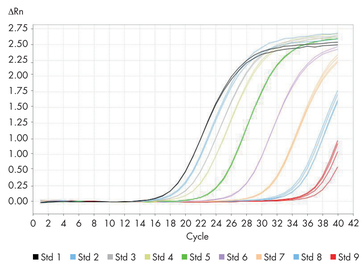
The Investigator Quantiplex Pro Kit PCR was evaluated for dynamic range. A male genomic DNA was serially diluted (based on A_{260}) from 200 ng/µl to 0.0005 ng/µl with 2 µl used per reaction. PCR setup was performed manually.

Data showed:

- A highly dynamic range from 200 ng/µl to 0.5 pg/µl
- High accuracy at low concentrations
- Consistent results



Standard curve using the Investigator Quantiplex Pro Kit. Control DNA was serially diluted from 200 ng/µl to 0.0005 ng/µl. DNA was amplified in duplicates with lower DNA concentrations in quadruplicates. Reaction efficiency and R^2 value are shown.



Amplification curves of the Investigator Quantiplex Pro Kit. Control DNA dilutions are shown. All runs were performed on an Applied Biosystems 7500 Real-Time PCR System for Human Identification.

Conclusions

The data presented demonstrate a novel, robust and precise quantification system that provides a very high sensitivity for male DNA, even in the presence of high amounts of female DNA. Furthermore, the assay provides a reliable assessment of a sample's integrity.

The Investigator Quantiplex Pro kit provides:

- Highly accurate and sensitive results for male DNA, even in high background of female DNA (up to 400,000:1)
- Precise assessment of DNA degradation
- High dynamic range from 200 ng/µl to 0.5 pg/µl
- Reliable information about inhibitors that correlates well with the STR results
- Fast-cycling technology and rapid quantification in 1 hour

The Investigator Quantiplex Pro assay's performance provides a better predictive ability for results with downstream STR assays. Optimized time-to-result allows more efficient integration into the forensic casework analysis workflow that can be further streamlined with QIAGEN's complementary automation for assay setup.

The applications presented here are for molecular biology applications. They are not intended for the diagnosis, prevention or treatment of a disease. 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.

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