**Introduction**

Forensic DNA laboratories are challenged by the requirement to provide results on the identity of genetic evidence within a very short time. Thus, in addition to crucial quality parameters, such as sensitivity and robustness, speed is an increasingly important feature of STR PCR assays. We have developed a set of next-generation Investigator Plus kits that combine all the critical features necessary for fast and reliable analysis of demanding forensic samples: ESSplex Plus (ESS, European Standard Set of loci), ESSplex SE Plus (including SE53), and Eplex Plus (Combined DNA Index System; CODIS).

Based on our fast-cycling PCR technology, we have introduced a novel reaction mix that allows the completion of a standard 30-cycle amplification in as little as 90 minutes. Using this protocol, well-balanced, full profiles can reliably be obtained with 100 pg of DNA template. All Plus assays are very robust regarding potential PCR inhibitors and can tolerate concentrations of up to 200 ng/mL humic acid, or up to 750 µM hematin. They provide a clean baseline without any dye artifacts. The combination of all aforementioned features helps to reduce the number of samples that need to undergo neorxenys, which further contributes to more streamlined and efficient laboratory workflows.

Utilizing the speed and robustness of the reaction mix, dedicated assays for direct amplification from database samples, e.g., blood or buccal cells on FTA paper are currently in development.

**Results: Fast amplification protocol**

The Investigator Plus PCR Kits make use of QIAGEN’s fast-cycling technology and utilize a very simple, fast, and robust PCR cycling protocol. Amplification is completed in about 90 minutes on a GeneAmp® PCR System 9700 with Gold-plated Silver 96-Well Block, run in max. mode. The protocol is generic and thus can be used for easy working with low template DNA samples.

Markers are well balanced within and across the 4 dye panels. Oligonucleotide design and purification methods have been optimized to completely remove any dye artifacts that would interfere with data analysis, especially when working with low template DNA samples.

**Methods: Fast multiplex PCR technology**

- Full Master Mix including Taq polymerase
- Use of purified and reprocessed Taq polymerase
- Multiplex PCR Reaction Buffer: Ammonium ions promote stable and efficient annealing of primers
- Factor MP stabilizes specifically bound primers
- Additive Gibb®3^® allows the DNA polymerase and primer to bind as a single complex
- Increases efficiency of primer annealing, reducing cycling time for highly complex multiplex assays
- Fast-set-up-robust amplification: Basic principles of the fast cycling mix were performed using a fast-cycling PCR thermocycler, then compared to standard cycling. Reproducibility of the Investigator PCR kit performance on different fast-cycling PCR thermocyclers is excellent. No differences were observed in terms of qualitative and quantitative PCR results. The same performance can be achieved using QIAGEN Investigator Plus kit.

**Sensitivity study of the Investigator ESSplex Plus Kit.** Standard DNA mix at different concentrations was amplified in 25 µl reaction volume, as shown.

**Conclusion**

The Investigator Plus kits provide following key features:

- New reaction mix based on QIAGEN fast multiplex technology
- Full master mix including Taq polymerase
- Short PCR protocol runtime of 90 min
- High inhibitor tolerance
- Highly sensitive using standard 30-cycle PCR protocol
- Clean baseline, free from dye artifacts
- In development: Direct amplification of database samples

**Results: Robustness**

The novel fast reaction mix has been optimized for inhibitor tolerance. It allows full DNA profiles to be obtained, even in the presence of very high concentrations of typical PCR inhibitors, such as hematin. This robustness helps to obtain DNA profiles from very challenging samples, and provides a technical basis for direct amplification from database samples, for example blood on FTA paper.

**Results: Sensitivity**

The Investigator Plus kits are very sensitive and show robust amplification over a wide range of DNA concentrations. Markers are well balanced within and across the 4 dye panels. Oligonucleotide design and purification methods have been optimized to completely remove any dye artifacts that would interfere with data analysis, especially when working with low template DNA samples.

**Sample & Assay Technologies**

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**Supplier Information**

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**Trademarks**

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**References**

- Scherer M., Müller D., Steeger B., Pakulla S., Breitbach M., Cornelius S., Fischer C., Bochmann L., Prochnow A., Schnibbe T., and Engel H.
- 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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