

Investigator® Plus kits: Fast, sensitive, and robust amplification of common STR standard set loci



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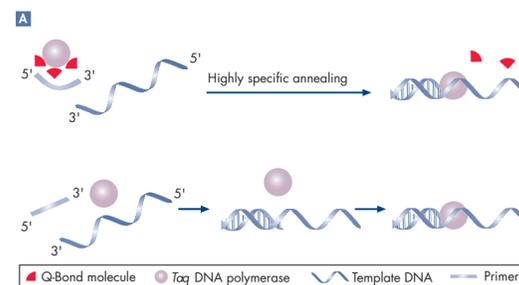
Introduction

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 SE33), and IDplex 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/μl 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 reanalysis, 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.

Methods: Fast multiplex PCR technology



Full Master Mix including Taq polymerase:

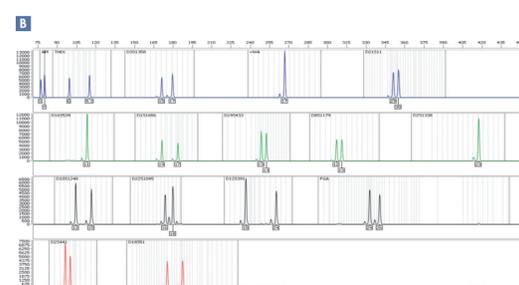
- Highest specificity due to stringent chemical hot-start of HotStarTaq® Plus Polymerase

Multiplex PCR Reaction Buffer:

- Ammonia ions promote stable and efficient annealing of primers
- Factor MP stabilizes specifically bound primers

Additive Q-Bond®:

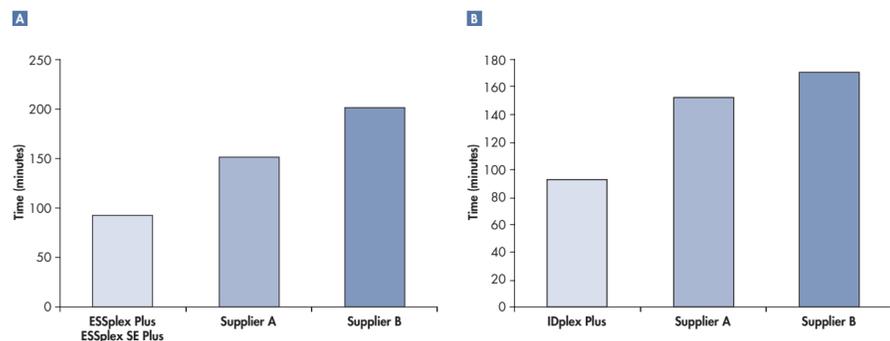
- allows the DNA polymerase and primer to bind as a single complex
- increases efficiency of primer annealing, reducing cycling time even for highly complex multiplex assays



Fast and robust amplification. A Basic principle of the fast-cycling multiplex PCR technology (upper panel) compared to standard cycling. B Electropherogram of the Investigator ESSplex Plus Kit using 500 pg Control DNA 9948. Analysis was performed on an Applied Biosystems 3500™ Genetic Analyzer. Allele assignment was performed using QIAGEN Investigator IDproof Software.

Results: Fast amplification protocol

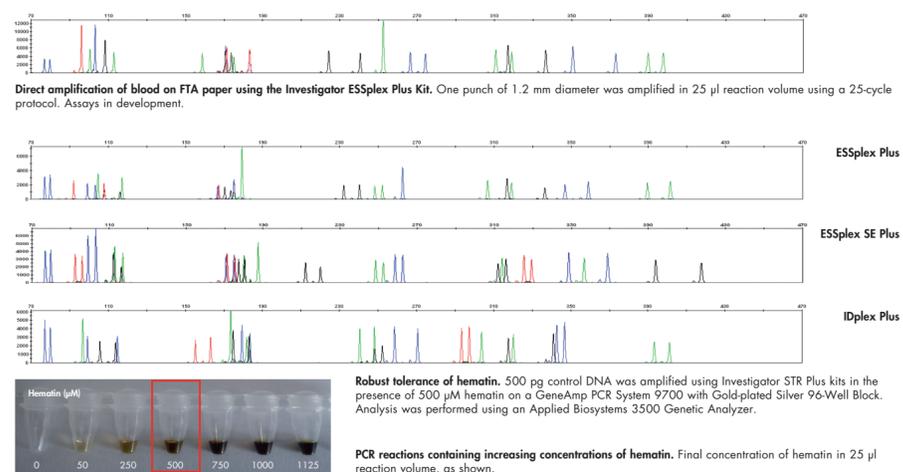
The Investigator Plus PCR Kits make use of QIAGEN's fast-cycling technology and utilize a very simple, fast, and robust PCR cycling protocol. Amplification is completed in about 90 minutes on a GeneAmp® PCR System 9700 with Gold-plated Silver 96-Well Block, run in max. mode. The protocol is generic and thus can be used for easy database-like samples, as well as for any type of casework sample containing low amounts of template DNA or inhibitors. In combination with optimized primer design, the new reaction chemistry allows a final extension step to be skipped completely, without causing the formation of -A products.



Fast PCR cycling of Investigator Plus kits. PCR Run time comparison. Effective run time is shown for A Investigator ESSplex Plus and ESSplex SE Plus and B Investigator IDplex Plus versus products from other suppliers covering equivalent marker sets. All PCRs used the GeneAmp PCR System 9700 with Gold-plated Silver 96-Well Block. All assays were run according to manufacturer's recommendations.

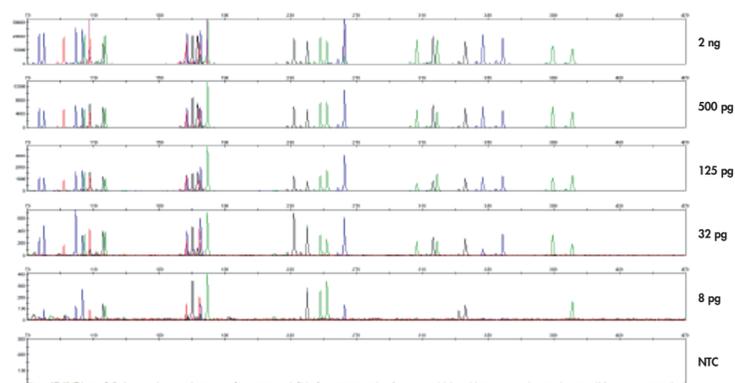
Results: Robustness

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.



Sensitivity study of the Investigator ESSplex Plus Kit. Control DNA XY5 at different concentrations was used as template. Samples were amplified using a GeneAmp PCR System 9700 with Gold-plated Silver 96-Well Block. Analysis was performed on an Applied Biosystems Genetic Analyzer 3500, equipped with a 36 cm 8-capillary array and POP4™ polymer. NTC: Notemplate control.

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



Overall time savings are >2 h per batch of samples compared to the fastest workflow solutions of other suppliers.

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

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