

The Investigator® ESSplex Plus Kit: Fast, sensitive, and robust amplification of the European Standard Set of loci



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Introduction

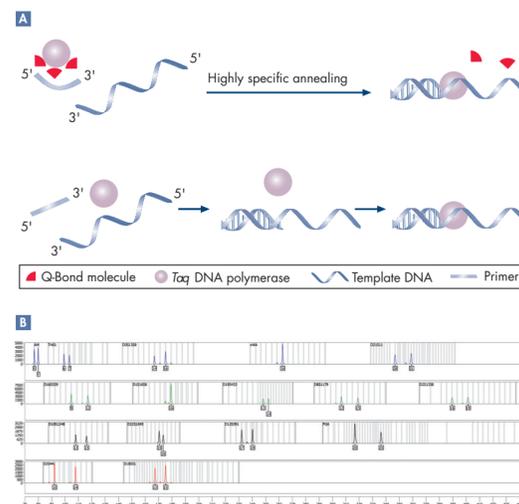
Forensic DNA laboratories need to provide results in a very short time. Therefore, in addition to crucial quality parameters (e.g., sensitivity and robustness), speed is an increasingly important feature of STR PCR assays. QIAGEN has developed the Investigator ESSplex Plus kit, which combines the features necessary for fast and reliable analysis of demanding forensic samples.

Based on our fast-cycling multiplex PCR technology, we have introduced a novel reaction mix that enables a standard 30 cycle amplification in ~90 minutes, setting a new level of speed in STR analysis. Well-balanced full profiles are reliably obtained with 100 pg of DNA template. Even a single genomic DNA copy gives rise to peak heights easily detectable with commonly used analysis thresholds.

The assay is very robust and can tolerate PCR inhibitors of up to 200 ng/µl humic acid or 1000 µM hematin without allelic dropout. The Investigator ESSplex Plus kit provides a clean baseline, without dye artifacts that may interfere with the analysis of low-copy-number samples.

These features help to reduce the number of samples that require reanalysis and contribute to more streamlined and efficient laboratory workflow.

Methods: Fast multiplex PCR technology



Fast multiplex technology provides fast cycling combined with high inhibitor tolerance.

Master Mix including Taq polymerase:

- High 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

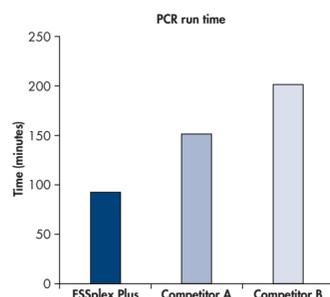
Q-Bond® additive:

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

Fast and robust amplification. A Basic principle of the Fast Cycling Multiplex PCR technology (top line) compared with standard cycling (bottom line). B Control DNA 9948 was amplified in the presence of 500 µM hematin. Analysis was performed on an AB 3500 Genetic Analyzer, data were analyzed using Investigator IDproof Software.

Results: Fast amplification protocol

The Investigator ESSplex Plus PCR Kit makes use of QIAGEN's fast cycling technology, enabling a simple, rapid, and robust PCR cycling protocol. Amplification is completed in 92 minutes on an ABI GeneAmp® PCR System 9700 Thermal Cycler with gold-plated silver block and run in Max Mode. The protocol is universal and 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 the final extension step to be missed out without the formation of -A products.



Comparison of PCR run times. Effective run time is shown for Investigator ESSplex Plus versus competitor products on an ABI GeneAmp PCR System 9700 Thermal Cycler with gold-plated silver block. All assays were run according to manufacturer's recommendations.

Results: Robustness towards PCR inhibitors

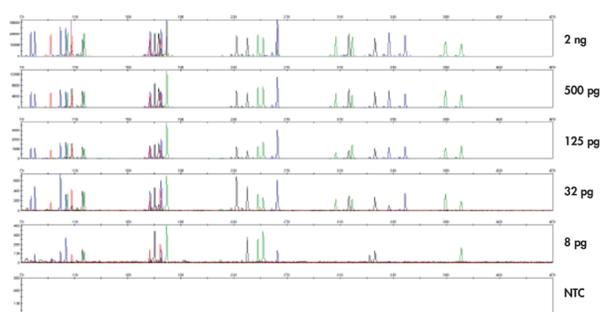
The novel Fast Reaction Mix has been optimized for inhibitor tolerance. It enables full DNA profiles to be obtained even in the presence of very high concentrations of typical PCR inhibitors. In turn, this helps to increase first round success rates with very challenging casework evidence and reduces workload created by reanalysis of samples.

STR marker	Humic acid						Hematin					Tannic acid					Indigo carmine							
	100 ng/µl	150 ng/µl	175 ng/µl	200 ng/µl	225 ng/µl	250 ng/µl	50 µM	250 µM	500 µM	750 µM	1000 µM	1125 µM	100 ng/µl	500 ng/µl	1000 ng/µl	1500 ng/µl	1750 ng/µl	2000 ng/µl	2 mM	4 mM	6 mM	8 mM	10 mM	12.5 mM
Amelogenin	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
TH01	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D3S1358	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
vWA	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D21S11	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D16S539	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D1S1656	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D19S433	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D8S1179	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D2S1338	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D10S1248	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D22S1045	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D12S391	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
FGA	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D2S441	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
D18S51	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

Overview of the Investigator ESSplex Plus inhibitor resistance. The assay was tested with 4 inhibitors (humic acid, hematin, tannic acid, and indigo carmine). 500 pg of Control DNA XY5 was used as template and PCR was performed under standard conditions. 100 RFU was used as the threshold for allele calling.

Results: Sensitivity

The Investigator ESSplex Plus kit is highly sensitive and shows 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 are 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. Different concentrations of Control DNA XY5 were used as a template. Samples were amplified on an ABI GeneAmp PCR System 9700 Thermal Cycler with gold-plated silver block. Analysis was performed on an AB Genetic Analyzer 3500, equipped with a 36 cm 8-capillary array and POP-4™ polymer. NTC: No-template control (pelase add to end of last sentence)

Conclusion

The Investigator ESSplex Plus Kit provides following key features:

- New reaction mix based on QIAGEN fast multiplex technology
- Full master mix including Taq polymerase
- Short PCR protocol runtime of 90 minutes
- High inhibitor tolerance
- High sensitivity using a standard 30-cycle PCR protocol
- Clean baseline, free from dye artifacts
- Streamlined and efficient workflow



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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