Development of a new multiplex PCR for global STR analysis

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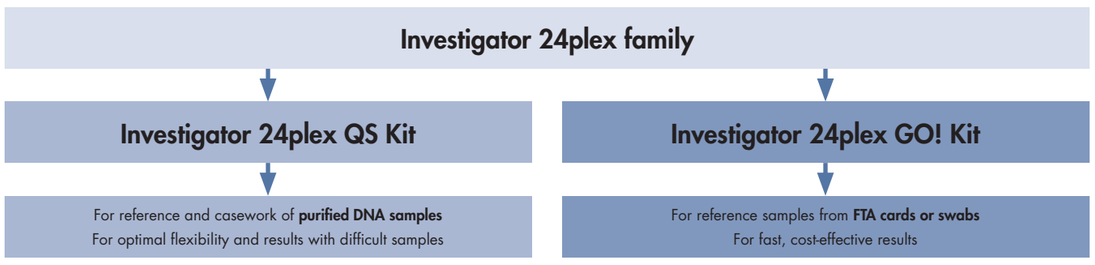
Introduction

The CODIS Core Loci Working Group has published recommendations to expand the CODIS core loci set in the United States. We developed an assay co-amplifying 23 markers according to the recommendations of the Working Group. The assay uses a 6-dye technology in order to keep the amplicon length of markers short, whilst avoiding marker overlap. The assay is based on a new PCR chemistry that ensures robust and fast PCR amplification with improved inhibitor resistance and easy handling.

There are two kit formats, covering:

- Purified DNA from casework and reference samples
- Direct amplification of reference samples, e.g., blood or buccal cells on FTA or swabs

Both kits contain a novel quality sensor (QS) system, used to evaluate amplification efficiency. The QS indicates whether the reaction has worked and discriminates between the presence of inhibitors or DNA degradation as a cause for the typical ski slope effect observed in STR profiles of challenging samples. This information then informs the most appropriate rework strategy.



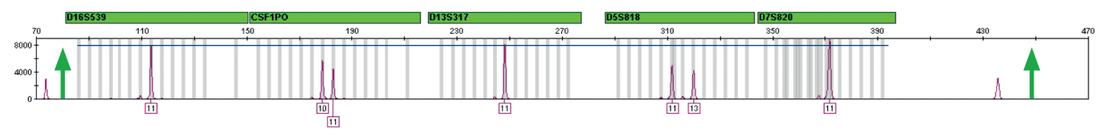
Quality sensor system

The Investigator 24plex QS Kits feature two internal PCR controls (QS1 and QS2) which provide useful information about the efficiency of the PCR and the presence of PCR inhibitors. The quality sensors are included in the primer mix and are amplified simultaneously with the polymorphic STR markers. The quality sensors are labeled with BTP and appear at fragment sizes of 74 bp (QS1) and 435 bp (QS2). For standard samples without degradation or inhibition, the QS monitors PCR amplification success.

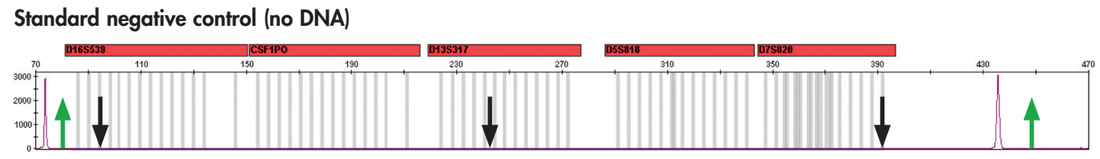
The quality sensor system distinguishes:

- Successful amplification
- Degraded DNA
- Inhibited DNA
- No DNA
- Failed PCR amplification
- Artificial sequences with no match to any known sequence

Standard sample (with DNA)



Standard negative control (no DNA)



Kit configuration

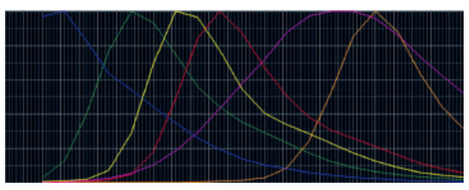
- All recommended CODIS expansion markers are included.
- Identical primers for Investigator 24plex QS and Investigator 24plex GO! Kits.
- BT6 enables high signal intensities, reduced amplicon length, and minimal allelic overlap.

		Codis 13		ESS		CODIS expansion		24plex
		13	15	12	15	19	22	23
1	SE33							
2	D1S1656							
3	D2S441							
4	D10S1248							
5	D12S391							
6	D22S1045							
7	D5S818							
8	D7S820							
9	D13S317							
10	CSF1PO							
11	TPOX							
12	D3S1358							
13	D8S1179							
14	D18S51							
15	D21S11							
16	FGA							
17	TH01							
18	vWA							
19	D16S539							
20	D2S1338							
21	D19S433							
22	DYS391							
23	Amelogenin							

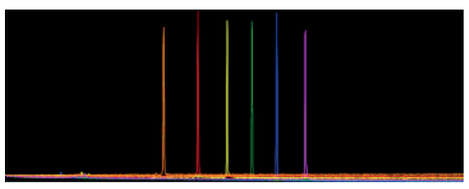
Section A (required)
Section B (recommended)
Consensus
Regional consensus

STR loci included. Meeting the recommendations of the CODIS Working Group.

A



B



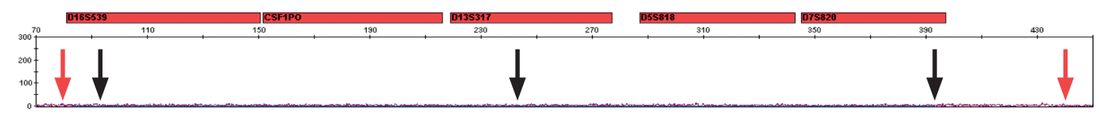
BT6: QIAGEN's new 6-color setup. A Intensity vs. pixel. B Intensity vs. scan.

Improved workflow

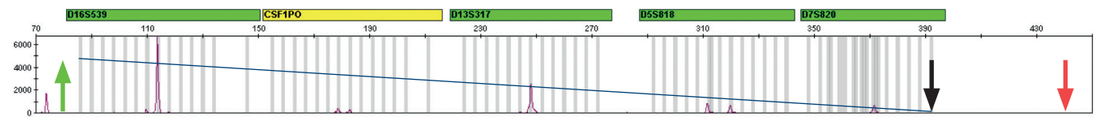
The QS system indicates the success of the PCR amplification. The absence of QS confirms PCR failure. Furthermore, the peak height ratio between QS1 and QS2 allows discrimination between the presence of inhibitors or DNA degradation as the cause.

	Allele peaks	QS1	QS2
Failed PCR	Absent	Absent	Absent
Inhibited sample	Ski slope profile	Present	Drop-down
Degraded sample	Ski slope profile	Present	Present

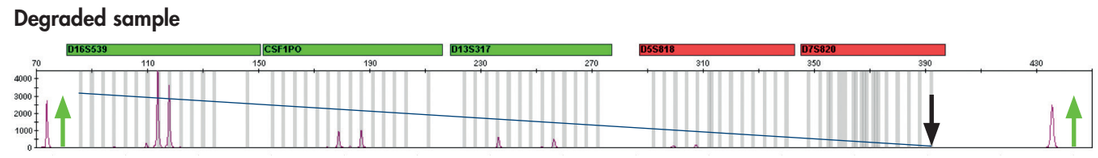
Failed PCR amplification



Inhibited sample



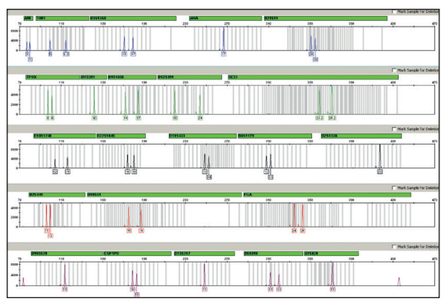
Degraded sample



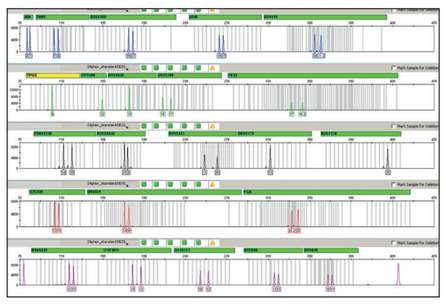
Amplification of typical sample types

Investigator 24plex QS allows robust and balanced amplification of purified DNA of casework and reference samples (A). The flat baseline facilitates the analysis of difficult and mixed samples. Investigator 24plex GO! provides direct amplification protocols for blood or buccal cells on FTA or similar paper and buccal swab crude lysates (B, C).

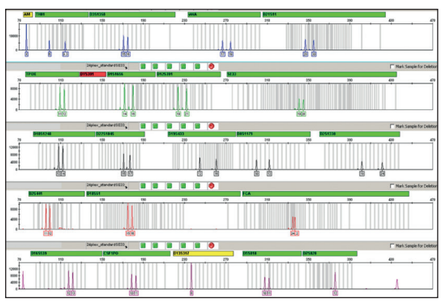
A



B



C



Electropherograms for different reference sample types processed using the Investigator 24plex Kit. All analyses were performed on an Applied Biosystems® 3500 Genetic Analyzer. A Investigator 24plex QS with Control DNA 9948. B Investigator 24plex GO! Kit with 2 µl buccal swab lysate; 27 cycle amplification. C Investigator 24plex GO! Kit with 1.2 mm punch of blood on FTA; 25 cycle amplification.

Conclusion

The Investigator 24plex QS Kit and Investigator 24plex GO! Kit feature:

- 23 markers recommended by the CODIS Core Loci Working Group
- Markers SE33 and DYS391
- Innovative quality sensors for more information and workflow optimization
- A new matrix for 6-color setup
- Full concordance with QIAGEN's existing STR GO! kits
- Allelic ladder with ~60 additional alleles
- Minimized allelic overlap for unambiguous results
- New reaction chemistry (FRM 2.0)
- High convenience and stability
- Rapid reaction speeds
- Very high inhibitor tolerance
- Convenient manual and automated pipetting
- Validated automated solutions for high- throughput needs

The applications presented here are intended 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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