Improving Sample Analysis and Interpretation Using STR Multiplex PCR Assays with a Novel Quality Sensor

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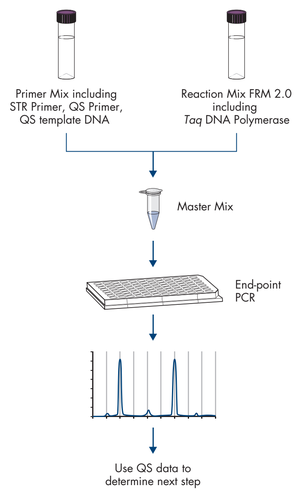
Introduction

The European STR standard set (ESS) of loci and new, expanded CODIS core loci set (as recommended by the CODIS Core Loci Working Group) have led to higher standardization and harmonization in STR analysis across borders. Various multiplex PCR assays have since been developed for the analysis of these sets that all meet high technical demands.

However, forensic analysts are often faced with difficult STR results and further questions. What was the reason that no peaks were visible in the electropherogram? Did the PCR fail? Was the DNA concentration too low? QIAGEN's newest Investigator STR kits contain a novel Quality Sensor (QS) that acts as an internal performance control and provides information useful for evaluating the amplification efficiency of the PCR. QS indicates whether the reaction has worked in general, and also allows discrimination between the presence of inhibitors or DNA degradation as a cause of the typical ski slope effect observed in STR profiles of such challenging samples. This information can be used to choose the most appropriate rework strategy.



The Quality Sensor distinguishes:

- Successful amplification
- Degraded DNA
- Inhibited DNA
- No DNA
- Failed PCR amplification

Kit Configurations

Investigator 24plex QS (NDIS approved): All recommended CODIS expansion markers for use with casework samples.

Investigator 24plex GO! (NDIS approved): All recommended CODIS expansion markers for use with reference samples.

- 6-color setup (Matrix BT6)
- Quality Sensor with two peaks (QS1, QS2) integrated into purple channel

Investigator ESSplex SE QS: European standard set of loci and SE33.

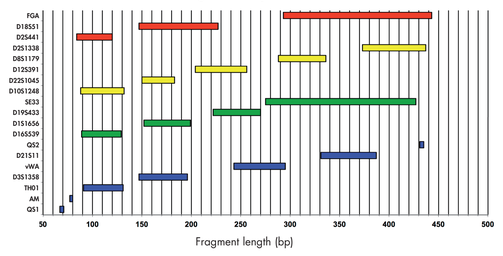
- 5-color setup (Matrix BT5)
- Quality Sensor with two peaks (QS1, QS2) integrated into blue channel

Investigator Argus X-12 QS: 12 X-chromosomal markers and D21S11.

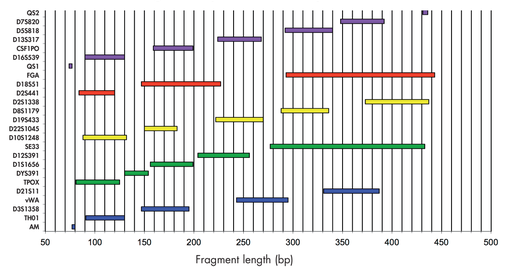
- 5-color setup (Matrix B5)
- Quality Sensor with one peak (QS1) integrated into blue channel

In all assays, the maximum fragment size in the allelic ladder is <450 bp and the QS is simultaneously amplified with the STR markers.

Investigator ESSplex SE QS



Investigator 24plex QS and 24plex GO!

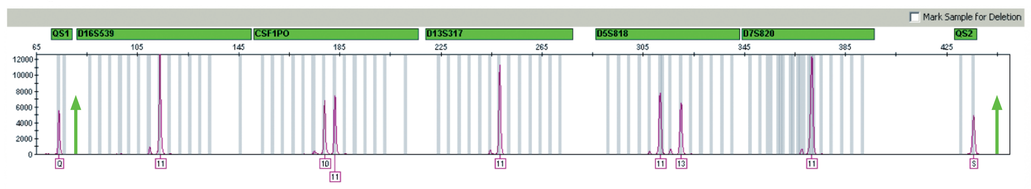


Marker arrangement. Marker ranges cover all alleles of the allelic ladder and are displayed according to the dye label used.

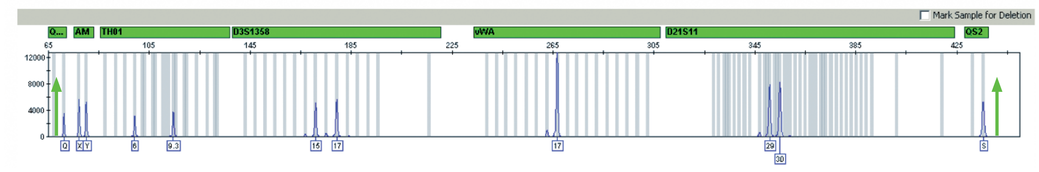
Quality Sensor — The Internal Performance Control

The Investigator 24plex QS, 24plex GO! and ESSplex SE QS Kits feature two internal PCR controls (QS1 and QS2) that provide information about the efficiency of the PCR and the presence of inhibitors. The QS primers and template are included in the primer mix. The QS is based on artificial sequences with no match to any known sequence (data not shown). It is amplified simultaneously with the polymorphic STR markers without affecting assay specificity or sensitivity. The QS has fragment sizes of ~70 bp (QS1) and ~435 bp (QS2). The high-molecular-weight product is far more sensitive to inhibition than the low-molecular-weight product. Dropdown or dropout of QS2 is therefore a clear indication of inhibition.

Investigator 24plex QS and 24plex GO! with QS in the purple channel



Investigator ESSplex SE QS with QS in the blue channel



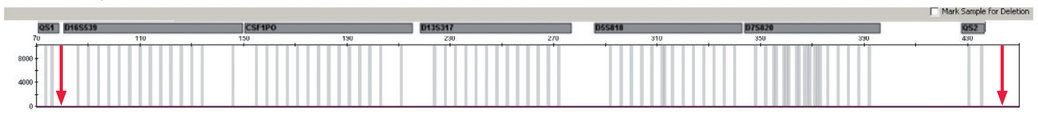
Quality Sensor. Amplification of 500 pg control DNA 9948, only channels with integrated QS are shown.

Data Interpretation

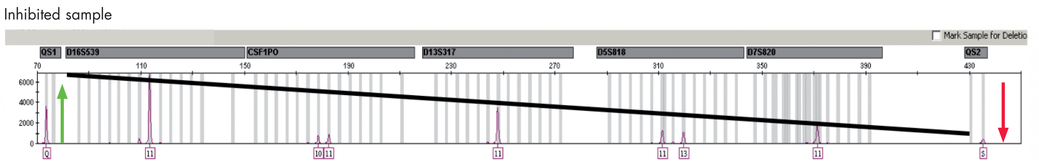
The QS indicates the success of the PCR amplification. The absence of QS confirms PCR failure. Furthermore, the peak height ratio between QS1 and QS2 allows discrimination between the presence of inhibitors or DNA degradation as the cause.

	Allele peaks	QS1	QS2
Failed PCR	Absent	Absent	Absent
Inhibited sample	Ski slope profile	Present	Dropdown
Degraded sample	Ski slope profile	Present	Present

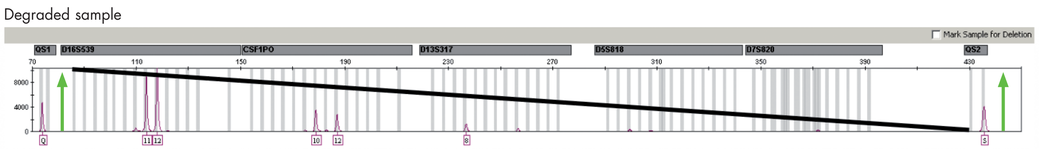
Failed PCR amplification



Inhibited sample



Degraded sample



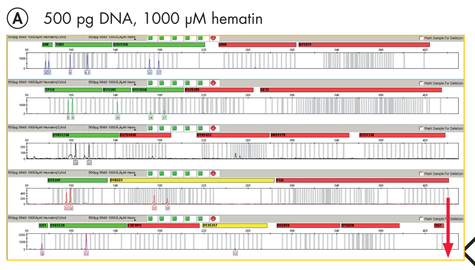
Quality Sensor function. Example data for the Investigator 24plex QS purple channel.

Improved Decision-making

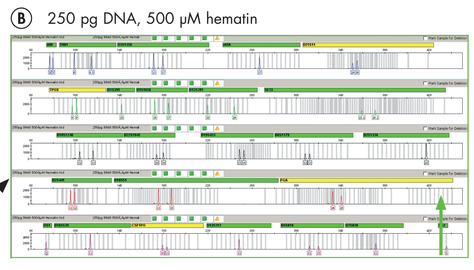
By enabling the clear distinction between inhibition and degradation as the cause of compromised data quality, the Quality Sensor can help to select the most appropriate rework strategy:

- Inhibition: Samples may be diluted for PCR or re-purified to reduce inhibitor load
- Degradation: More template DNA may be used to push high molecular weight markers above threshold.

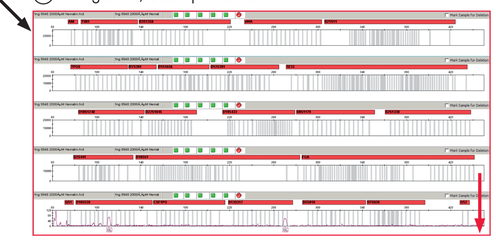
Ⓐ 500 pg DNA, 1000 µM hematin



Ⓑ 250 pg DNA, 500 µM hematin



Ⓒ 1 ng DNA, 2000 µM hematin



Example for a rework of an inhibited sample. Ⓐ 500 pg control DNA was amplified in the presence of 1000 µM hematin. All high molecular markers and QS2 dropped out and only 19 of 40 expected alleles are detected, indicating inhibition. Ⓑ Rework strategy as suggested by the QS information. Sample input was reduced to overcome inhibition, a full profile and QS2 were recovered. Ⓒ Alternative rework strategy if sample was falsely rated as degraded (not having QS information). Increased template and inhibitor load led to complete failure.

Conclusions

Today, forensic and paternity-testing laboratories are faced with ever-increasing workloads. The innovative Quality Sensor improves the workflow by reducing costs and increasing efficiency, productivity and quality of laboratory operations:

- Confirms good PCR performance
- Confirms negative results
- Differentiates between inhibited and degraded samples
- Suggests proper rework strategy

Enabling the generation of these additional, valuable data for quality control and performance checks, Investigator PCR STR kits containing the Quality Sensor further streamline the workflow of casework samples without affecting PCR performance. Furthermore, they also help to improve the first-time success rates for reference samples that are not quantified in advance in a direct amplification setup.

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

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Sample to Insight