Application Note

Increase analysis efficiency and rework of low-quality samples with the automated Quality Sensor flagging in GeneMarker®HID software

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Introduction

Amplification inhibitors and degradation can complicate forensic profile analysis by producing incomplete genotypes from casework samples. Common amplification inhibitors such as indigo dyes from denim and haemoglobin are thought to decrease *Taq* DNA polymerase by binding to the active site of the enzyme. Degradation starts at the time of death due to degradative enzymes and also from environmental conditions, such as UV irradiation (1). QIAGEN® provides a 2-fragment internal control in the Investigator® Quality Sensor which saves time and improves workflow by providing information dealing with failed PCR amplification, absence of DNA, inhibited DNA and degraded DNA (2). Analysts use the quality sensor peaks to provide essential information of problematic samples, based on the presence/absence or peak height ratios of the Q and S fragments in combination with the peak heights of the sample profile.

Table 1. Summary of visual interpretation for the range of Q and S peaks in problematic samples

Interpretation	Peaks
Confirmed successful PCR amplification but absence of DNA	QS1 and QS2 appear at similar heights. No sample allele peaks appear.
Failed PCR amplification	Lack of QS1 and QS2. No sample allele peaks appear.
Inhibited DNA	QS1 with normal peak height and QS2 with decreased peak height can be seen if inhibitors are affecting PCR. Sample allele peaks for the markers show decreasing height towards the larger markers.
Degraded DNA	QS1 and QS2 appear at similar heights. Sample shows allele peaks for the STR loci with decreasing height towards the larger STR loci.

Features

While these Quality Sensors improve the laboratory workflow by improving the understanding of results and aiding decision making, analysts need to make a subjective judgment on these markers to use them. GeneMarkerHID software provides further efficiencies by providing parameters to automatically flag samples that break any of the rules for Q and S quality sensor peaks.

Figure 1 shows the qS flagging features of the GeneMarkerHID electropherogram display.

- A qS flag alerts the analyst
- Mouse-over provides information on the rule fired
- Mouse click displays a pop-up message with the details about the rules fired

This example fired the rule for exponential degradation of peak height with the increasing fragment size.

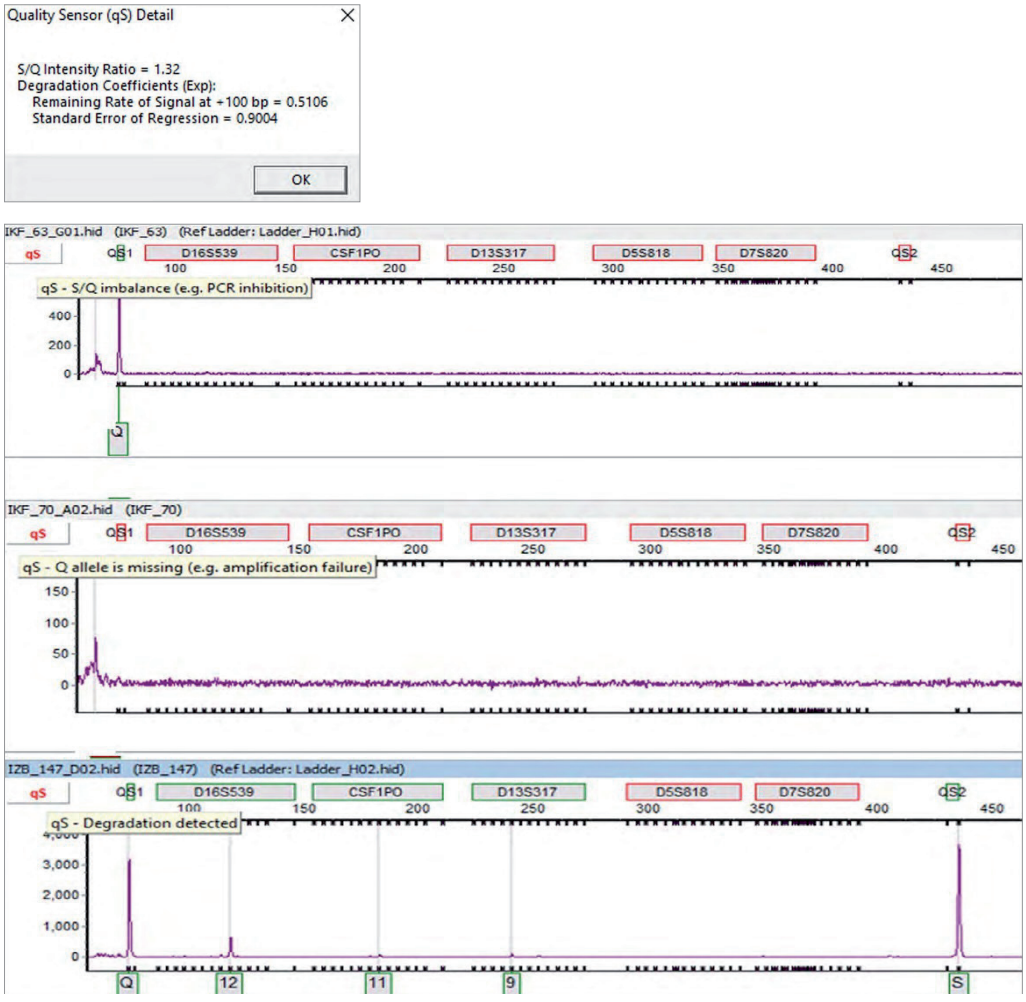

Figure 1. Quality sensor (qS) flagging features of the GeneMarkerHID electropherogram.

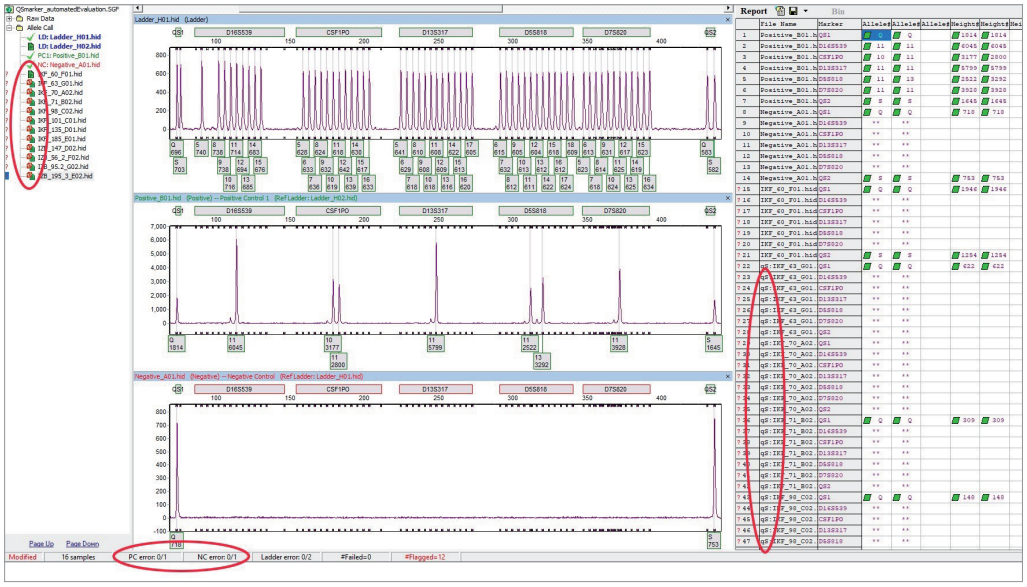

Figure 2. Overview of the main analysis screen in GeneMarkerHID software. Details include positive and negative control concordance, qS flagging of samples, the electropherogram, and the exported report table.

The main GeneMarkerHID analysis screen (Figure 2) displays

- Positive and negative control concordance
  Note: The negative control has no error in the summary bar at the bottom of the screen when Q and S fragments are detected.
- qS flag of appropriate samples in the file name tree at left
- The electropherogram
- The report table that is exported to the laboratory LIMS system

Conclusion

GeneMarker provides additional time savings and avoids potential human bias when evaluating samples that have unacceptable peak heights or decreasing peak height.

Acknowledgements

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References

1. Butler, J.M. (2012) *Advanced Topics in Forensic DNA Typing: Methodology*, 1st ed. Cambridge, MA: Academic Press.
2. Kraemer, M., Prochnow, A., Bussmann, M., Scherer, M., Peist, R. and Steff, C. (2017) Developmental validation of QIAGEN Investigator 24plex QS Kit and Investigator 24plex GO! Kit: Two 6-dye multiplex assays for the extended CODIS core loci. FSIGenetics **29**, 9.

Ordering Information

Product	Contents	Cat. no.
Investigator 24plex QS Kit (100)	Primer Mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder 24plex, DNA size standard 24plex (BTO) and nuclease-free water	382415
Investigator 24plex QS Kit (400)	Primer Mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder 24plex, DNA size standard 24plex (BTO) and nuclease-free water	382417
Investigator 24plex GO! Kit (200)	Primer Mix, Fast Reaction Mix 2.0 including Taq DNA polymerase, Control DNA, allelic ladder 24plex, DNA size standard 24plex (BTO)	382426
Investigator 24plex GO! Kit (1000)	Primer Mix, Fast Reaction Mix 2.0 including Taq DNA polymerase, Control DNA, allelic ladder 24plex, DNA size standard 24plex (BTO)	382428
Investigator 26plex QS Kit (100)	Primer Mix, Fast Reaction Mix 3.0 including Taq DNA Polymerase, Control DNA, allelic ladder 26plex, and nuclease-free water	382615
Investigator 26plex QS Kit (400)	Primer Mix, Fast Reaction Mix 3.0 including Taq DNA Polymerase, Control DNA, allelic ladder 26plex, and nuclease-free water	382617
Related Products		
Investigator ESSplex SE QS Kit (100)	Primer Mix, Fast Reaction Mix 2.0 including Taq DNA Polymerase, Control DNA, allelic ladder, ESSplex SE QS, DNA size standard 550 (BTO), nuclease-free water	381575
Investigator ESSplex SE QS Kit (400)	Primer Mix, Fast Reaction Mix 2.0 including Taq DNA Polymerase, Control DNA, allelic ladder, ESSplex SE QS, DNA size standard 550 (BTO), nuclease-free water	381577

Please contact us at **info@softgenetics.com** for more information and a time-limited trial of GeneMarkerHID software.

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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