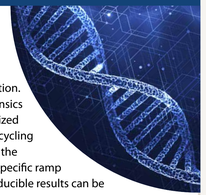
Evaluation of the QIAamplifier® 96 Thermal Cycler

Madeline Roman, PhD, Signature Science, LLC • **Josh Abernathy, MSFS**, QIAGEN • **Bryan Davis, BS**, QIAGEN

INTRODUCTION



A typical forensic DNA workflow involves amplification of STRs, and the quality and accuracy of STR results is affected by the thermal cycler used for amplification. Multiplex STR assays used in forensics are thoroughly tested and optimized to be run under specific thermal cycling conditions. User manuals specify the compatible thermal cyclers and specific ramp rates under which reliable, reproducible results can be obtained.

The purpose of this study was to test the compatibility of a newly available thermal cycler, the QIAamplifier® 96 (QIAGEN), with STR multiplexes used for human identification. Sensitivity, accuracy, reproducibility, and inhibition studies were performed using the Investigator® 24plex QS kit (QIAGEN), and results from the QIAamplifier® 96 were compared to results from the Veriti® 96-Well Thermal Cycler (Thermo Fisher Scientific). In addition, compatibility with direct PCR was tested using the Investigator® 24plex GO! Kit.

The QIAamplifier® 96 features a high-speed thermoblock, heated smart lid, and a linear gradient ability. In this study, data produced using this new thermal cycler is compared to data produced on a Veriti 96-Well Thermal Cycler in order to provide confidence that the thermal cyclers produce similar quality data when used with highly-optimized forensic STR multiplexes. It is expected that other STR chemistries not tested in this study would show similar results on the QIAamplifier 96® as compared with the Veriti.

OBJECTIVES

- To test the compatibility of a new, open end-point PCR thermal cycler from QIAGEN with STR chemistries used for human identification.
- To directly compare results from Investigator 24plex QS obtained on the QIAamplifier to results obtained on an alternative thermal cycling system, the Veriti 96-Well Thermal Cycler.
- To assess sensitivity, reproducibility, and inhibition differences between the thermal cyclers by analyzing allele recovery, peak heights, peak height ratios, and intra-color balance of STR electropherograms.

Figure 1: The QIAamplifier® 96, a new thermal cycling system for end-point PCR from QIAGEN. It is compatible with a range of plasticware and consumables already used in forensic laboratories.

MATERIALS & METHODS

Instrumentation: Thermal cyclers were programmed according to the Investigator 24plex QS Kit Handbook using 29 cycles. The ramp rate on the QIAamplifier® 96 was set to 4 °C/s, and the heated lid was set to 104 °C. On the Veriti, a 100% ramp rate (3.9 °C/s) and heated lid temperature of 105 °C were used. For 24plex GO! experiments, 27 cycles were used. Capillary electrophoresis was performed on a 3500xL using the manufacturer's recommended injection conditions for each kit.

Sensitivity: Two male DNA samples were amplified in triplicate at 1 ng, 0.5 ng, 0.25 ng, 0.125 ng, and 0.063 ng.

Reproducibility: The 24plex QS Kit positive control DNA (0.5 ng) and four NTCs were amplified with 12 replicates on each thermal cycler.

Inhibition: Positive control DNA (0.5 ng) was spiked with hematin at 10 µM, 20 µM, and 30 µM concentrations. Samples were amplified in quadruplicate on each thermal cycler.

Direct PCR: Three buccal swabs were directly amplified in triplicate on each thermal cycler using a 27-cycle amplification program.

All experiments were performed at the Signature Science, LLC Forensic Laboratory, and data analysis was performed at QIAGEN.

RESULTS

Sensitivity

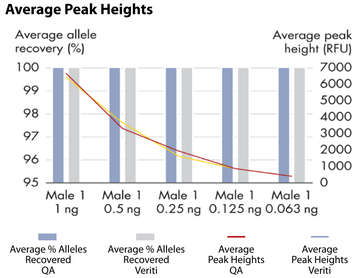


Figure 2: Average allele recovery and peak heights for Male 1 in the sensitivity study. QA = QIAamplifier 96. Complete profiles were recovered at all dilutions across both thermal cyclers. No differences in peak height average were observed using a two-tailed F test.

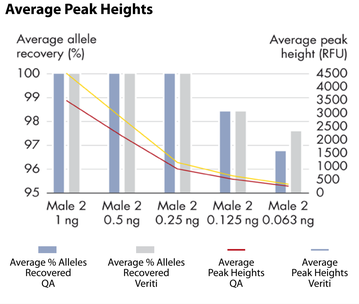


Figure 3: Average allele recovery and peak heights for Male 2 in the sensitivity study. QA = QIAamplifier 96. Dropout of alleles was observed at 0.125 ng input and below for both thermal cyclers. Peak height averages fell to 65% for both systems at an input of 0.063 ng. No differences in peak height average were observed using a two-tailed F test.

Reproducibility

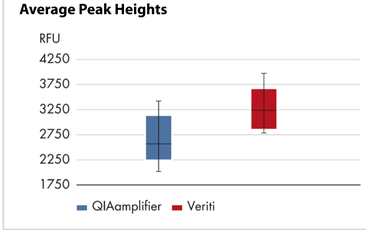


Figure 4: Average peak heights from 12 positive control replicates on the QIAamplifier and Veriti systems. No significant difference was detected using a two-tailed F test.

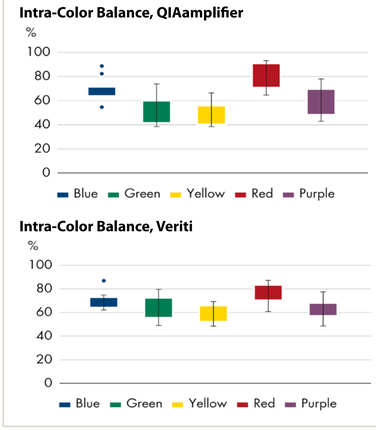


Figure 5: Intra-color balance for positive control DNA (12 replicates) on the QIAamplifier (Figure 4a) and Veriti (Figure 4b). No significant differences were observed using the two-tailed F statistic.

Inhibition

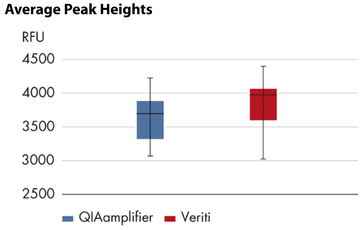


Figure 6: Average peak heights from positive control samples spiked with hematin (10 µM, 20 µM and 30 µM), run in quadruplicate. All samples resulted in 100% allele calls across all concentrations of hematin on both thermal cyclers. There was no significant difference between the QIAamplifier and Veriti data (two-tailed F statistic). Average peak height ratios ranged from 83-90% with an average of 87% for all samples. No significant differences were observed for intra-color balance (two-tailed F statistic).

Direct PCR

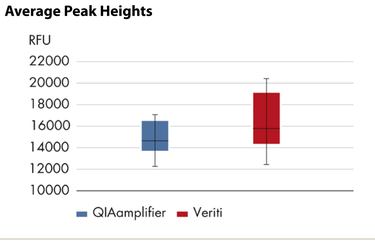


Figure 7: Average peak heights for two buccal swabs amplified in triplicate on each thermal cycler with the Investigator 24plex GO! kit. All replicates resulted in 100% allele recovery, and the average peak height was 91% for all samples with no significant difference between QIAamplifier and Veriti data (two-tailed F test). No significant differences were observed for intra-color balance (two-tailed F statistic).

CONCLUSIONS

- The QIAamplifier 96 is compatible with DNA typing using the Investigator 24plex QS and Investigator 24plex GO! kits.
- Comparable results were obtained on the QIAamplifier 96 and Veriti systems, with no significant differences in allele recovery, peak heights, peak height ratios, or intra-color balances between the two thermal cyclers.
- Though additional STR chemistries were not tested in this study, it is likely that they could generate highly comparable results when amplified using the QIAamplifier 96.

ACKNOWLEDGEMENTS & CONTACT

The authors would like to thank Leslie Parke of Signature Science, LLC for her help in facilitating these experiments.

Signature Science, LLC
8329 N. Mopac Expressway
Austin, TX 78759

Maddie Roman
mroman@signaturescience.com

QIAGEN
19300 Germantown Road
Germantown MD 20874

Bryan Davis
Bryan.Davis@qiagen.com

Josh Abernathy
Josh.Abernathy@qiagen.com

An application note has been published with the data from this study and is available at QIAGEN.com.