Application Note

QIAamplifier® 96 compatibility testing with Investigator® 24plex QS and Investigator® 24plex GO! Kits

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Introduction

The accuracy of STR analyses, an essential component of human identification (HID) efforts, is dependent on the efficacy of PCR amplification of the target loci. In addition to requiring quality amplification, these analyses must ideally be performed quickly and in an automated fashion to most effectively support the work of forensic laboratories. QIAGEN offers the Investigator® 24plex QS Kit (QIAGEN) and the Investigator 24plex GO! Kit, which provide robust, multiplex amplification of the CODIS (Combined DNA Index System) core loci, the ESS (European Standard Set) markers, as well as SE33, DYS391, D2S1338, D19S433 and Amelogenin.

The newly released, high-performance QIAamplifier® 96 thermal cycler system has the potential to further streamline the STR analysis workflow. The QIAamplifier 96 is an end-point PCR instrument featuring a 96-well, 0.2 mL aluminum block with gradient functionality and is controlled through a 7" touchscreen user interface (Figure 1). It is suitable for any end-point PCR application in molecular biology workflows such as genotyping, target detection, semi-quantitative gene expression, reverse transcription and multiplex PCR.

The QIAamplifier 96 includes a high-speed thermoblock, a high-performance smart lid, a user-specific list of quick start programs, easy programming and a linear gradient tool.

The QIAamplifier 96 is also an open system, meaning it is compatible with a wide range of consumables and plasticware. For additional details on these features, please review the QIAamplifier 96 User Manual.

The purpose of this application note is to confirm the compatibility of the QIAamplifer 96 with HID STR amplification chemistry. We further compared the performance of the QIAamplifier 96 to the Applied Biosystems® Veriti® 96-Well Thermal Cycler, which represents an alternative cycling system.

Figure 1. The QIAamplifier 96 Thermal Cycler.

Materials and Methods

Thermal cycler programs

The Investigator 24plex QS Kit was used to assess the compatibility of STR amplification chemistry with the QIAamplifier 96. A 29-cycle amplification program, the times and temperatures of which are described in the Investigator 24plex QS Kit Handbook, was used. The QIAamplifier 96 was set to a ramp rate of 4°C/sec and a heated cover temperature of 104°C. The 24plex QS Kit was also applied to the Applied Biosystems Veriti 96-Well Thermal Cycler, which used a 100% ramp rate and heated cover temperature of 105°C. The Veriti 96-well thermal cycler has a published average maximum block ramp rate of 3.9°C/sec.

In addition, samples prepared from three different buccal swabs using the Investigator 24plex GO! Kit were tested on both thermal cyclers. Each swab was prepared and amplified at 27 cycles, in triplicate, as described in the Investigator 24plex GO! Handbook. A total of 18 samples were amplified across both thermal cyclers.

Assessment parameters

Three parameters were used to assess the efficacy of the STR reactions, as indicated below: reproducibility, sensitivity and inhibition.

Reproducibility

12 replicates of the Investigator 24plex QS Kit positive control and 4 negative template controls (NTCs) were amplified across both thermal cyclers, for a total of 32 samples. A target input amount of 0.5 ng was used for the positive controls.

Sensitivity

Two male genomic samples were used to create a 5-point dilution series (1 ng, 0.5 ng, 0.25 ng, 0.125 ng and 0.063 ng) and were amplified in triplicate across both thermal cyclers, for a total of 60 samples.

Inhibition

0.5 ng of Investigator 24plex QS Kit positive control was spiked with one of three final concentrations of hematin (10 µM, 20 µM and 30 µM) in the amplification reactions. Each sample was amplified in quadruplicate across both thermal cyclers for a total of 24 samples.

Capillary electrophoresis and data analysis

All samples were injected and electrophoresed on an Applied Biosystems 3500xL Capillary Electrophoresis instrument (with Data Collection v3.1), following the recommended injection and run conditions outlined in the respective amplification kit handbooks. Subsequent data analysis was performed in GeneMapper® ID-X v1.6 using the standard panels, bins, and stutter filters. Samples amplified with 24plex QS were analyzed at the following peak amplitude thresholds: 60 relative fluorescence units (RFU; Blue), 65 RFU (Green), 65 RFU (Yellow), 65 RFU (Red), 60 RFU (Purple) and 60 RFU (Orange). Samples amplified with 24plex GO! were analyzed at a peak amplitude threshold of 150 RFU for all dye channels with a 0.2 Global Cutoff Filter.

The average peak heights for each sample were determined by dividing the total RFU for all STR fragments by the total number of true donor alleles recovered, excluding the QS. The average peak height ratio (intra-locus balance) at a heterozygous locus was determined by dividing the lower peak height of the two alleles by the higher peak height.

Intra-color balance was determined by dividing the lowest average peak height of a locus in a dye channel by the highest average peak height of another locus in the same channel. The average peak height of a locus was calculated by dividing the total RFU of the true donor alleles at that locus by two. DYS391 and the QS were excluded from this calculation.

The average allele recovery was calculated by counting the number of true donor alleles detected above the peak amplitude threshold and dividing by the total number of expected alleles for that sample.

Results

All genotypes obtained across both thermal cyclers were 100% concordant with the reference genotypes. The results were further examined for average peak heights, average peak height ratio (intra-locus balance), intra-color balance, and, in the case of the sensitivity analysis, the average allele recovery.

Reproducibility

All expected alleles were recovered from all replicates amplified across both thermal cyclers. The average RFUs of the Investigator 24plex QS Kit positive controls, along with their distributions, are shown in Figure 2. When the averages for each replicate were compared and analyzed using a two-tailed probability of the F statistic, no significant difference was observed. All replicates across both platforms averaged a peak height ratio of 88%, with a range of 84–91%.

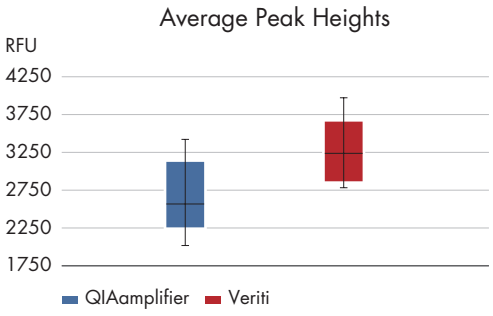

Figure 2. Box and whisker plot of the average peak heights of the Investigator 24plex QS positive control replicates.

The intra-color balance distributions for each thermal cycler are plotted in Figures 3 and 4. The lowest observed average intra-color balance was 49% (yellow dye channel in Figure 3) and the highest observed average intra-color balance was 80% (red dye channel in Figure 3). When the averages for each dye channel were compared and analyzed using a two-tailed probability of the F statistic, no significant difference was observed.

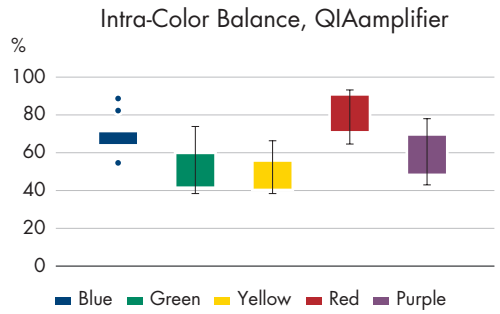

Figure 3. Box and whisker plot of the intra-color balance of the Investigator 24plex QS positive control amplified using QIAamplifier 96.

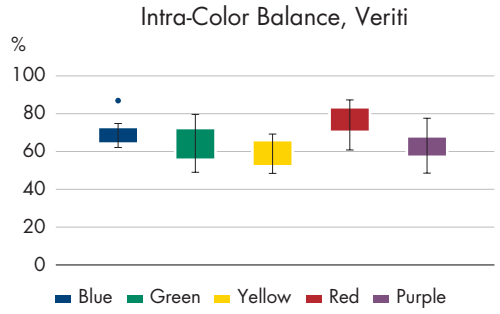

Figure 4. Box and whisker plot of the intra-color balance of the Investigator 24plex QS positive control amplified using Veriti.

The NTCs used in this study did not produce amplified DNA. Moreover, the QIAamplifier 96 baseline results were similar to those produced by the Veriti.

Sensitivity

Male 1

The average allele recovery and the average peak height of all replicates for Male 1 used in the analysis of sensitivity are displayed in Figure 5. All dilution points recovered 100% of alleles across both platforms. As expected, the peak heights decreased in a linear fashion as the target input amounts decreased. When the peak height averages for each replicate were compared and analyzed using a two-tailed probability of the F statistic, no significant difference was observed.

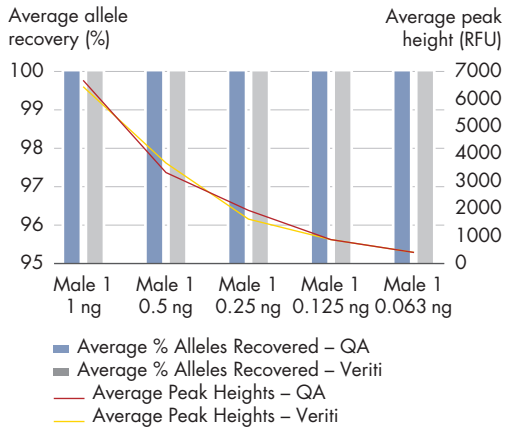

Figure 5. Average allele recovery and average peak height for Male 1 in the analysis of sensitivity (QA = QIAamplifier 96).

Average peak height ratios, as expected, decreased as the peak heights decreased and the total input amounts of DNA reached or exceeded the stochastic level of the kit. At 0.063 ng input, the average peak height ratio for both thermal cyclers was 69%.

The intra-color balance ranged from 45% (yellow channel, 0.063 ng input, Table 2) to 94% (red channel, 1ng input, Table 1). Overall, the intra-color balance was consistent between the thermal cyclers and a two-tailed probability of the F statistic found no significant differences between the data points.

Male 2

The average allele recovery and the average peak height of all replicates for Male 2 used in the analysis of sensitivity are displayed in Figure 6. Allelic drop-out was observed beginning at 0.125 ng input. As expected, the peak heights decreased in a linear fashion as the target input amounts decreased. When the peak height averages for replicates with complete allele recovery (i.e., ≥ 0.250 ng) were compared and analyzed using a two-tailed probability of the F statistic, no significant difference was observed.

Table 1. Intra-color balance for Male 1 in the analysis of sensitivity using the QIAamplifier 96

QIAamplifier Input amount	Blue	Green	Yellow	Red	Purple
1 ng	71%	49%	55%	94%	81%
0.5 ng	73%	57%	52%	78%	81%
0.25 ng	70%	53%	46%	83%	78%
0.125 ng	65%	50%	55%	73%	62%
0.063 ng	56%	62%	48%	66%	47%

Table 2. Intra-color balance for Male 1 in the analysis of sensitivity using the Veriti

Veriti Input amount	Blue	Green	Yellow	Red	Purple
1 ng	70%	63%	61%	81%	84%
0.5 ng	72%	56%	56%	74%	77%
0.25 ng	70%	62%	52%	81%	85%
0.125 ng	59%	74%	60%	70%	68%
0.063 ng	73%	51%	45%	63%	60%

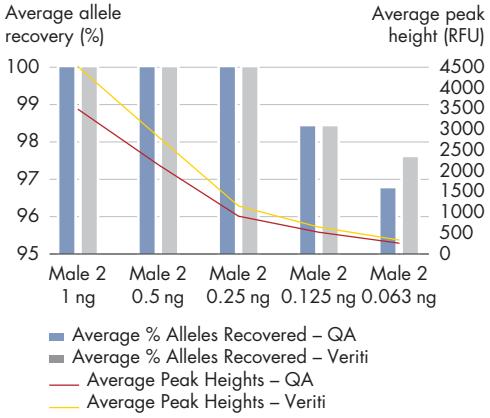

Figure 6. Average allele recovery and average peak height for Male 2 in the analysis of sensitivity (QA = QIAamplifier 96).

Consistent with the results for Male 1, the average peak height ratios for Male 2 decreased as the peak heights decreased and the total amount of input DNA reached or exceeded the stochastic level of the kit. At 0.063 ng input, the average peak ratio for both thermal cyclers was 65%.

The intra-color balance ranged from 33% (yellow channel, 0.063 ng input, Table 4) to 91% (red channel, 1 ng input, Table 3). As allelic drop-out increased, the average intra-color balance decreased. Overall, the intra-color balance was consistent between the thermal cyclers and a two-tailed probability of the F statistic found no significant difference between samples with complete allele recovery.

Table 3. Intra-color balance for Male 2 in the analysis of sensitivity using the QIAamplifier 96

QIAamplifier Input amount	Blue	Green	Yellow	Red	Purple
1 ng	59%	62%	53%	91%	76%
0.5 ng	61%	65%	52%	88%	80%
0.25 ng	58%	52%	47%	68%	75%
0.125 ng	66%	46%	37%	85%	68%
0.063 ng	48%	43%	41%	57%	57%

Table 4. Intra-color balance for Male 2 in the analysis of sensitivity using the Veriti

Veriti Input amount	Blue	Green	Yellow	Red	Purple
1 ng	67%	73%	48%	82%	81%
0.5 ng	67%	75%	51%	81%	87%
0.25 ng	54%	73%	42%	77%	74%
0.125 ng	62%	74%	39%	73%	84%
0.063 ng	42%	64%	33%	57%	54%

Inhibition

All of the positive control samples obtained 100% allele recovery with all levels of inhibitor tested across both thermal cyclers. The average peak height distributions are plotted in Figure 7. No significant difference was observed when the averages for each replicate were compared and analyzed using a two-tailed probability of the F statistic. The average peak height ratios averaged 87% for all samples, with a range of 83–90%.

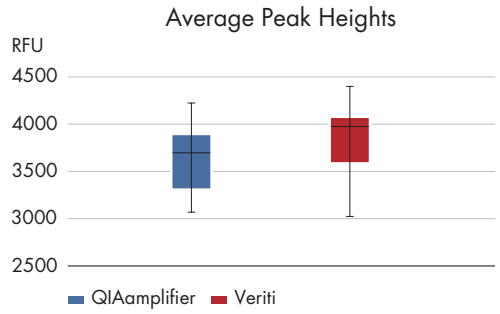

Figure 7. Box and whisker plots of the average peak heights of Investigator 24plex QS positive controls spiked with varying levels of hematin.

The intra-color balance distributions for each thermal cycler are plotted in Figures 8 and 9. The lowest observed average intra-color balance was 49% (yellow dye channel, Figure 8) and the highest average observed was 77% (red channel, Figure 8). When the averages for each dye channel were compared and analyzed using a two-tailed probability of the F statistic, no significant difference was observed.

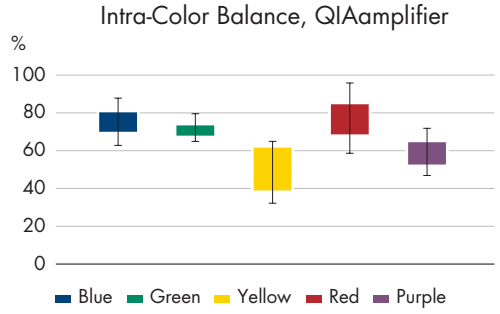

Figure 8. Box and whisker plot of the intra-color balance for Investigator 24plex QS positive controls spiked with varying levels of hematin amplified on the QIAamplifier 96.

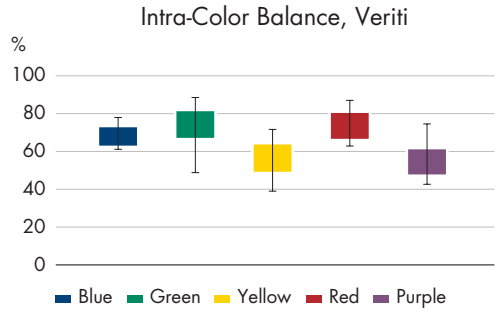

Figure 9. Box and whisker plot of the intra-color balance for Investigator 24plex QS positive controls spiked with varying levels of hematin amplified on the Veriti.

Direct Amplification

All replicates amplified across both thermal cyclers obtained 100% allele recovery. The average peak height distributions are plotted in Figure 10. No significant difference was observed when the averages for each replicate were compared and analyzed using a two-tailed probability of the F statistic. The average peak height ratios averaged 91% for all samples, with a range of 88–92%.

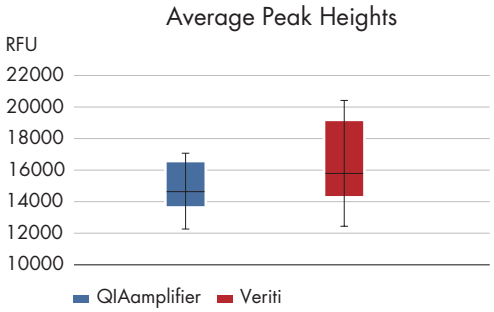

Figure 10. Box and whisker plot of the average peak heights for buccal swabs amplified with Investigator 24plex GO!.

The intra-color balance distributions for each thermal cycler are plotted in Figures 11 and 12. The lowest observed average intra-color balance was 43% (yellow dye channel, Figure 11) and the highest average observed was 87% (green channel, Figure 12). When the averages for each dye channel were compared and analyzed using a two-tailed probability of the F statistic, no significant difference was observed.

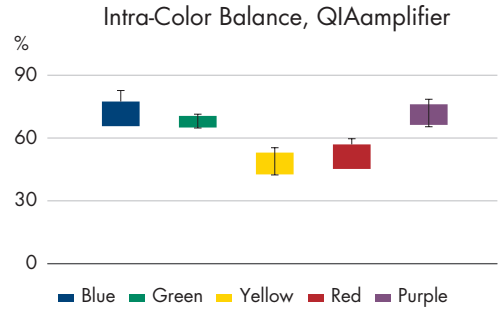

Figure 11. Box and whisker plot of the intra-color balance for buccal swabs amplified with Investigator 24plex GO! using the QIAamplifier 96.

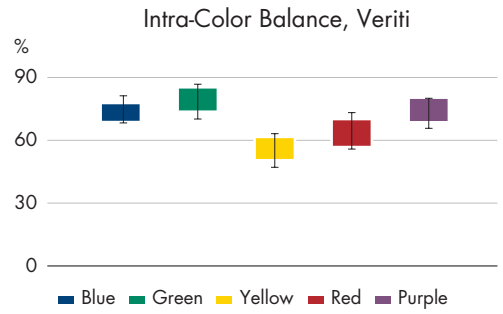

Figure 12. Box and whisker plot of the intra-color balance for buccal swabs amplified with Investigator 24plex GO! using the Veriti.

Conclusions

Our results indicate that the QIAamplifier 96 is compatible with the STR amplification chemistry of the Investigator 24plex QS Kit and the Investigator 24plex GO! Kit. STR amplification using both kits performed well on the QIAamplifier 96 thermal cycler platform, as well as the Veriti system. We observed no significant differences in peak heights, allele recovery, peak height ratio, or intra-color balances between these platforms as assessed using a two-tailed probability of the F statistic. We also observed no new reproducible STR artifacts. We propose that additional human identification STR kits could generate highly comparable data when amplified on a QIAamplifier 96.

Summary

Use of the QIAamplifier 96 thermal cycler provides:

- Compatibility with the STR amplification chemistry of the Investigator 24plex QS Kit and the Investigator 24plex GO! Kit
- Efficient, reproducible STR amplification, competitive with other thermal cycler platforms
- An open thermal cycler system for flexibility of chemistries and plasticware

Acknowledgments

We thank Leslie Parke and Madeline Roman of Signature Science, LLC, 8329 MoPac Expressway, Austin, TX 78757 for their help in facilitating these experiments.

Ordering Information

Product	Contents	Cat. no.
QIAamplifier 96 (115 V)	Fast cycling, high performance thermocycler with a 96-well block and a color touchscreen interface for end-point PCR requirements. 115 V power specification	9002990
QIAamplifier 96 (220 V)	Fast cycling, high performance thermocycler with a 96-well block and a color touchscreen interface for end-point PCR requirements. 220 V power specification	9002991
Investigator 24plex QS Kit (100)*	Primer Mix, Fast Reaction Mix 2.0 including *Taq* DNA Polymerase, Control DNA, allelic ladder 24plex, DNA size standard 24plex (BTO), and nuclease-free water	382415
Investigator 24plex GO! Kit (200)*	Primer Mix, Fast Reaction Mix 2.0 including *Taq* DNA polymerase, Control DNA, allelic ladder 24plex, and DNA size standard 24plex (BTO)	382426
Related Products		
Matrix Standard BT6 (50)	Matrix standard for 6-FAM, BTG, BTY, BTR2, BTP, and BTO, for Applied Biosystems 3500 Genetic Analyzers	386224
Investigator STR GO! Lysis Buffer (200)	Lysis buffer for 200 swab samples	386516
Investigator STR GO! Punch Buffer (200)*	Lysis buffer for 200 samples of epithelial cells on paper	386526

* Larger kits sizes available.

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