

# Functional performance comparison of legacy and refreshed 3730 Series DNA Analyzers

## Introduction

Released in 2002, the 3730 and 3730xl DNA Analyzers have been dependable instruments for high-throughput Sanger sequencing and fragment analysis by capillary electrophoresis. With age comes the inevitable need to upgrade the instruments as parts become obsolete. Here we provide sequencing and fragment analysis data demonstrating that refreshed instruments have functional performance comparable to legacy instruments. Existing performance specifications are met or exceeded in all tests performed.

## Methods

Sequencing performance in terms of Q20 contiguous read length (CRL) was evaluated using three legacy instruments and three refreshed instruments. Sequencing data were collected using Applied Biosystems™ POP-7™ Polymer (Cat. No. 4335615), 3730 DNA Analyzer 48-Capillary Array, 36 cm (Cat. No. 4331247), 3730xl DNA Analyzer 96-Capillary Array, 50 cm (Cat. No. 4331246), and BigDye™ Terminator v3.1 Sequencing Standard (Cat. No. 4336943). Data for two consecutive injections were collected for each array configuration. Sequencing data were viewed with Applied Biosystems™ Sequence Scanner Software v2.0 for Q20 CRL performance.

Fragment analysis performance in terms of sizing precision and accuracy was evaluated using data collected during system verification activities. Data for the Applied Biosystems™ DS-33 GeneScan™ Installation Standard with GeneScan™ 600 LIZ™ Size Standard v2.0 (Cat. No. 4376911) were collected using POP-7 Polymer (Cat. No. 4335615) and 3730 DNA Analyzer 48-Capillary Array, 36 cm. Two legacy instruments were compared to eight refreshed instruments. A single injection was collected for each array configuration. Sizing precision and accuracy were determined using Applied Biosystems™ GeneMapper™ Software 6 (Cat. No. 4475074).

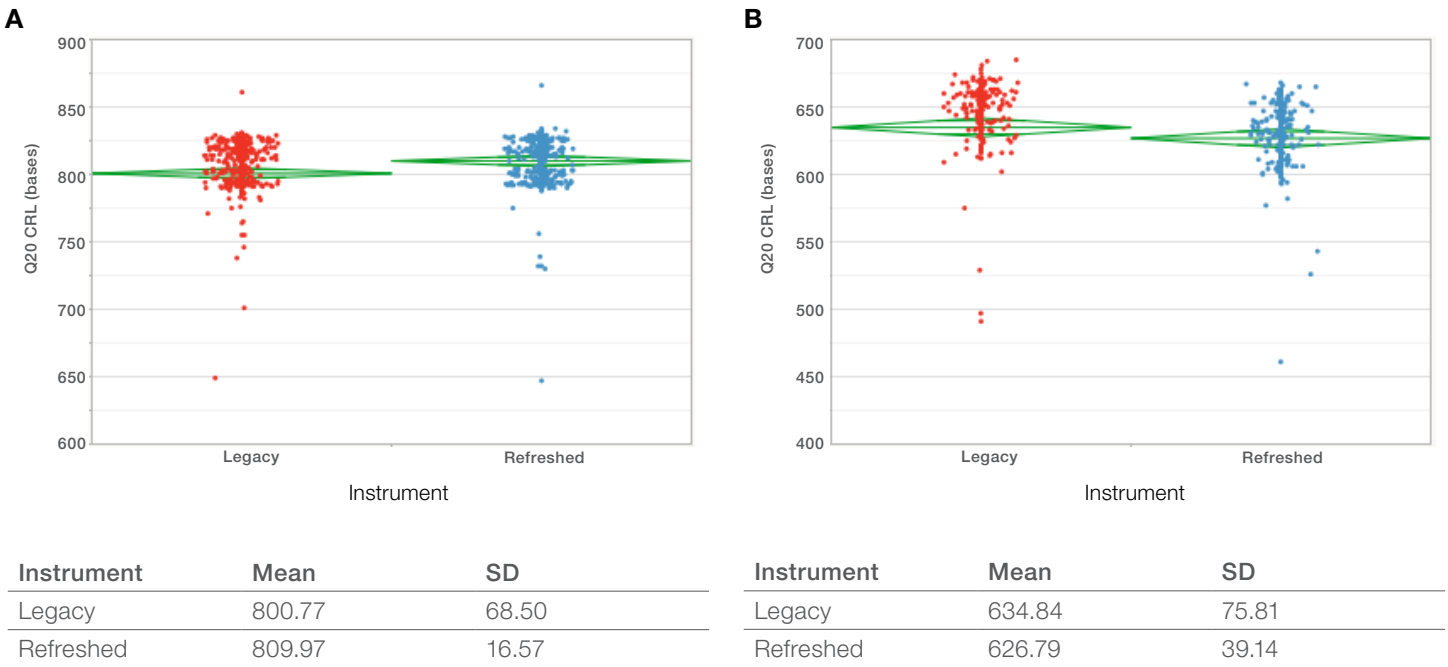
Statistical analysis was performed using JMP™ Software 13.1.0. Differences between group means were tested using one-way analysis of variance (ANOVA). Mean and standard deviation (SD) were reported by the software as part of this analysis.

## Results

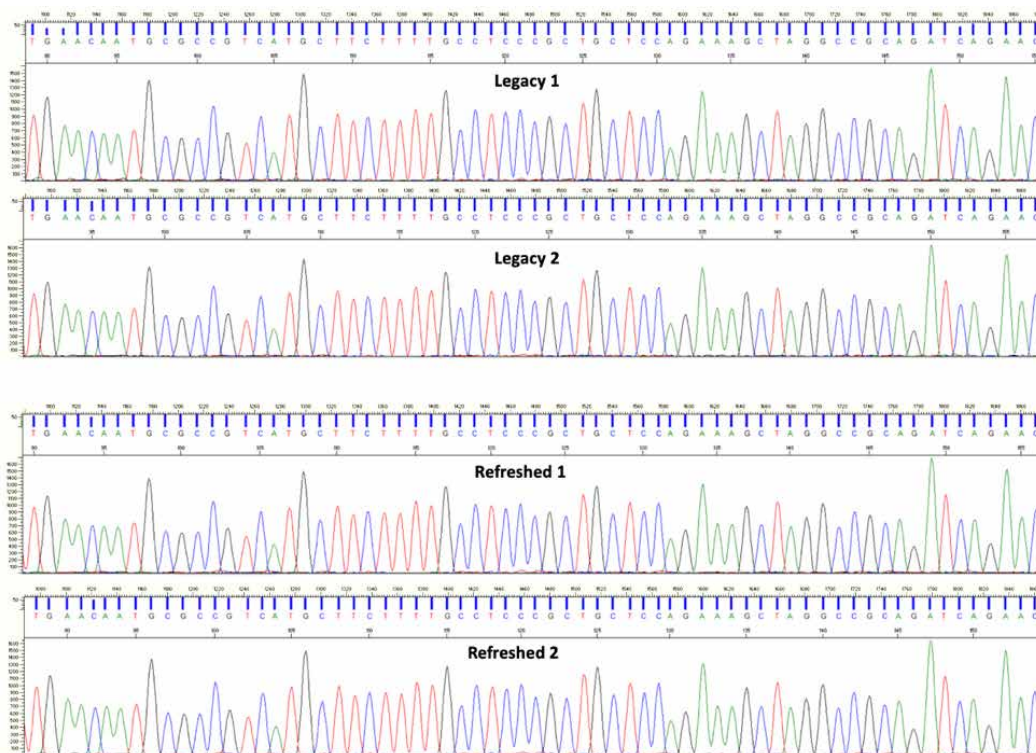
### Sequencing performance

Refreshed instruments perform similarly to legacy instruments with all tested array configurations, meeting

or exceeding Q20 CRL specifications. Figures 1 and 2 summarize these performance data and provide example sequencing traces.



**Figure 1. One-way ANOVA of Q20 CRL performance by instrument.** No statistically significant difference in performance is observed between data from legacy and refreshed 3730 Series DNA Analyzers with the (A) 96-capillary or (B) 48-capillary array configuration. In charts showing one-way ANOVA in this paper, the line in the center of each green diamond represents the group mean. The vertical span of each green diamond represents the 95% confidence interval for the mean of each group.

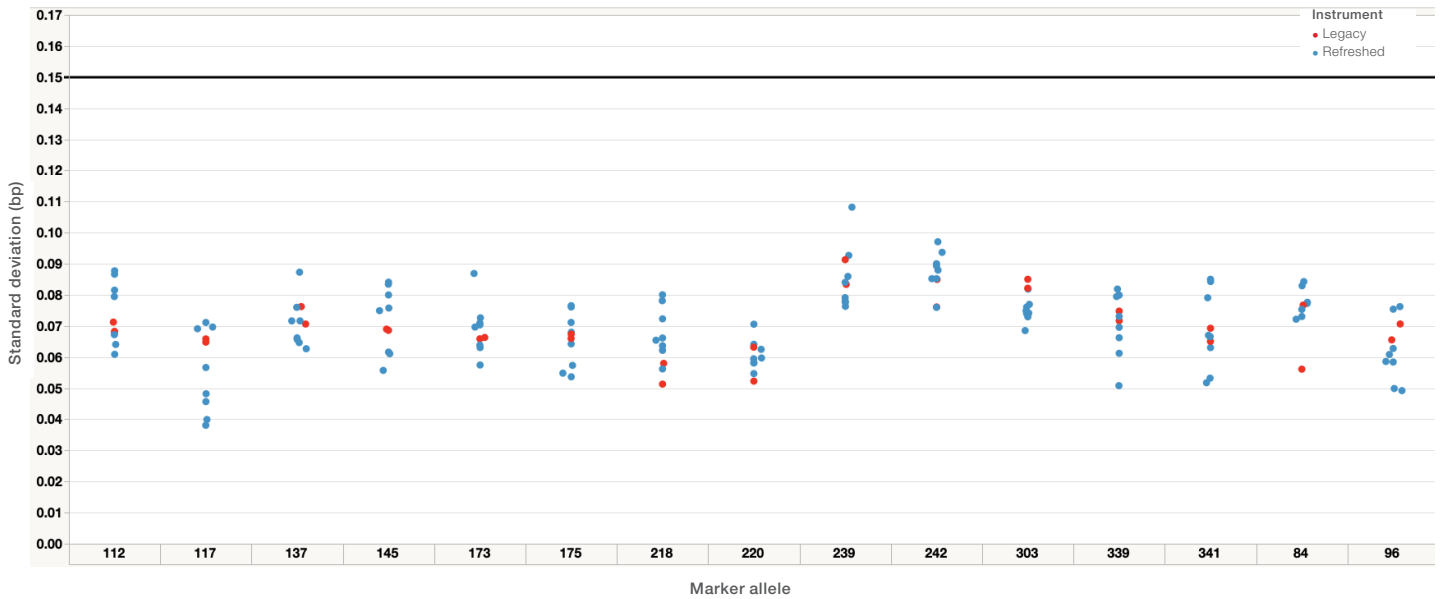


**Figure 2. Representative sequencing traces from two legacy and two refreshed 3730 Series DNA Analyzers with 48-capillary arrays.**

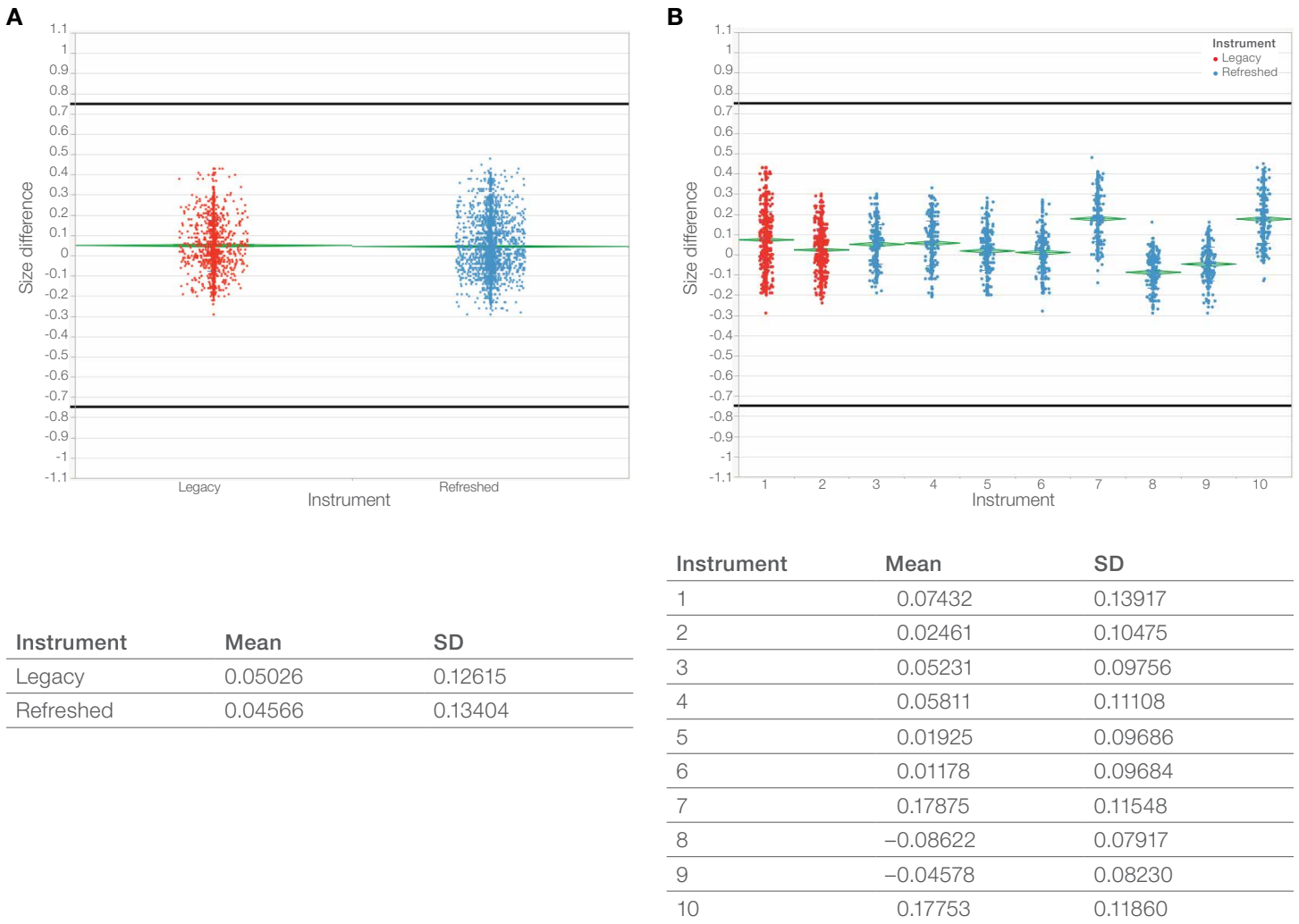
### Fragment analysis performance

Comparisons of fragment analysis data are from the first injection. The largest marker fragment in the DS-33 GeneScan Installation Standard is ~340 bp long. A passing run is one in which all 15 markers produce a standard deviation of  $\leq 0.15$  bp. The specification requires that 90% of all injections pass. All runs passed the  $\leq 0.15$  bp

sizing precision specification for all markers in the DS-33 GeneScan Installation Standard. For both legacy and refreshed instruments, the 90% specification is met. Figure 3 shows that sizing precision is similar across legacy and refreshed instruments.



**Figure 3. Comparison of sizing precision.** Legacy and refreshed 3730 DNA Analyzers meet the passing standard deviation of  $\leq 0.15$  bp. Fragment analysis data were collected with ten instruments: two legacy instruments and eight refreshed instruments.



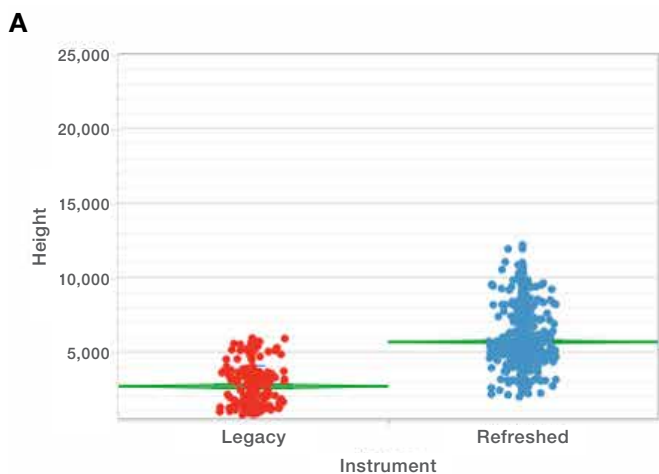
**Figure 4. One-way ANOVA of sizing accuracy.** Sizing accuracy is similar for legacy and refreshed 3730 DNA Analyzers. Comparisons are shown (A) by group of two legacy or eight refreshed instruments and (B) by individual instrument.

Additionally, it is important to demonstrate sizing accuracy across multiple instruments. Sizing accuracy tells us that a marker is properly sized within a genotyping bin. Sizing accuracy is determined by calculating the size difference relative to the expected nominal marker. A size difference of 0 shows that an allele's size is exactly the expected size of the nominal marker. Figure 4 shows that sizing accuracy is comparable across legacy and refreshed instruments.

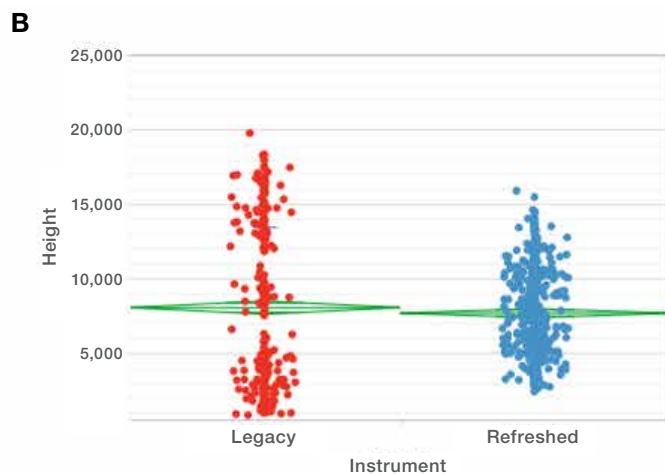
### Signal intensity

The refreshed 3730 DNA Analyzer was upgraded with a solid-state laser. This change led to an increase in the 6-FAM™ (522 nm) signal, the bluest dye in our standard fragment analysis suite (DS-33 standard, G5 dye set). Much of the 6-FAM emission spectrum is cut off in the legacy 3730xl DNA Analyzer, making it weak compared to the other dyes. The fragment analysis data show that the 6-FAM signal is enhanced, approximately 2-fold, on refreshed systems compared to legacy instruments, bringing it near equivalence with the other dyes, which were unaffected in the new system.

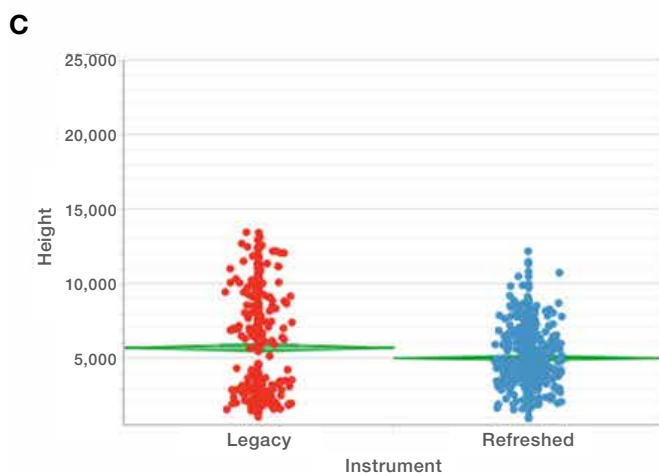
Figure 5 shows the peak heights on the legacy and refreshed 3730 DNA Analyzers for the 6-FAM™, NED™, PET™, and VIC™ dyes used in the DS-33 GeneScan™ Installation Standard.



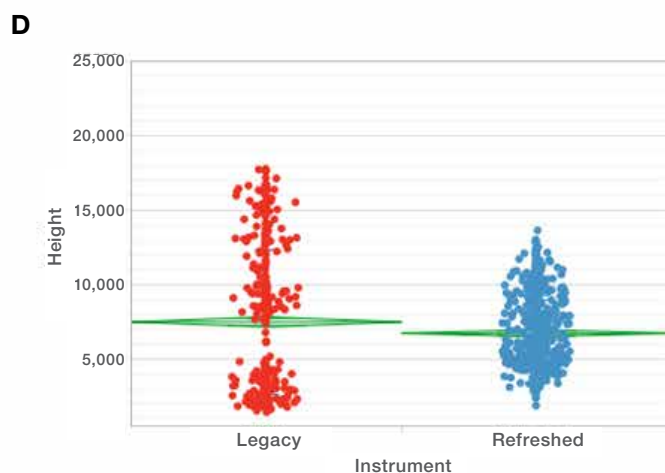
Instrument	Mean	SD
Legacy	2,687.19	1,364.05
Refreshed	5,675.72	1,817.48



Instrument	Mean	SD
Legacy	8,075.36	5,389.51
Refreshed	7,697.68	2,693.93



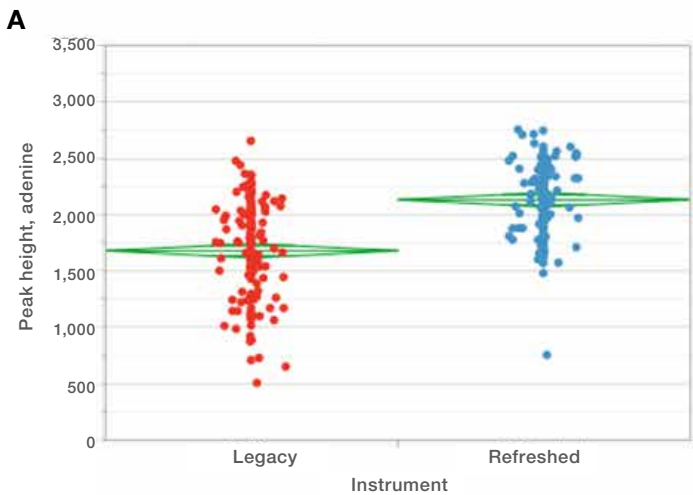
Instrument	Mean	SD
Legacy	5,670.03	3,445.28
Refreshed	4,979.22	1,789.00



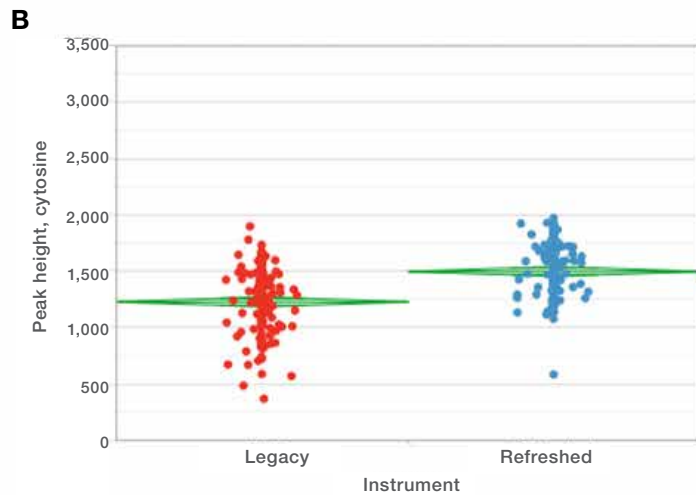
Instrument	Mean	SD
Legacy	7,503.45	4,876.59
Refreshed	6,742.99	2,224.00

Figure 5. One-way ANOVA of peak heights. Data are shown for (A) 6-FAM, (B) NED, (C) PET, and (D) VIC dyes.

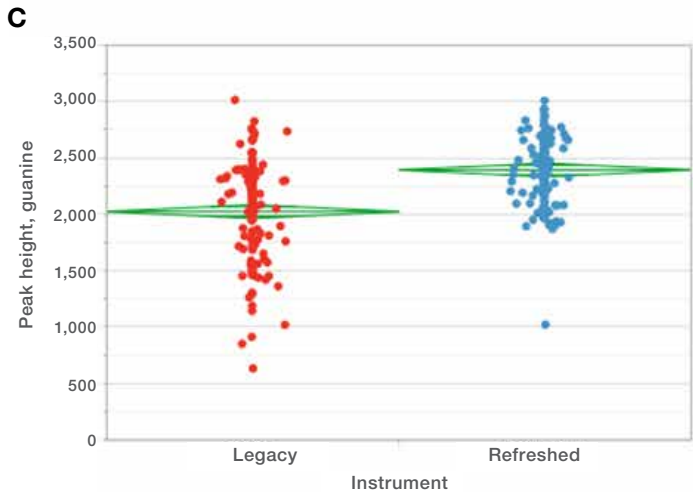
For comparison, peak heights of sequencing analysis dye signals for adenine, cytosine, guanine, and thymine bases are displayed in Figure 6 for the 48-capillary array, 36 cm configuration. A similar experiment is shown in Figure 7 for the 96-capillary array, 50 cm configuration.



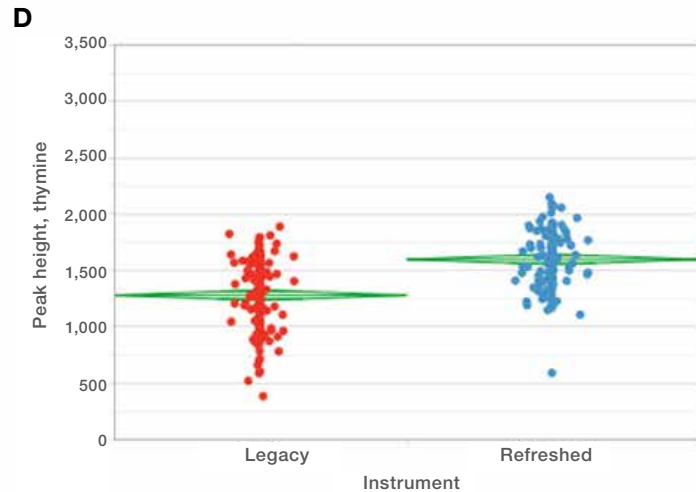
Instrument	Mean	SD
Legacy	1,679.82	446.24
Refreshed	2,132.17	311.14



Instrument	Mean	SD
Legacy	1,227.92	287.61
Refreshed	1,498.30	225.31

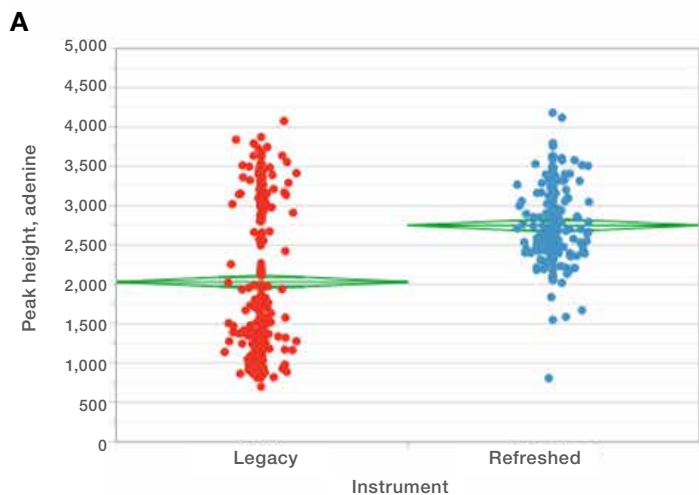


Instrument	Mean	SD
Legacy	2,024.32	434.01
Refreshed	2,393.07	284.60

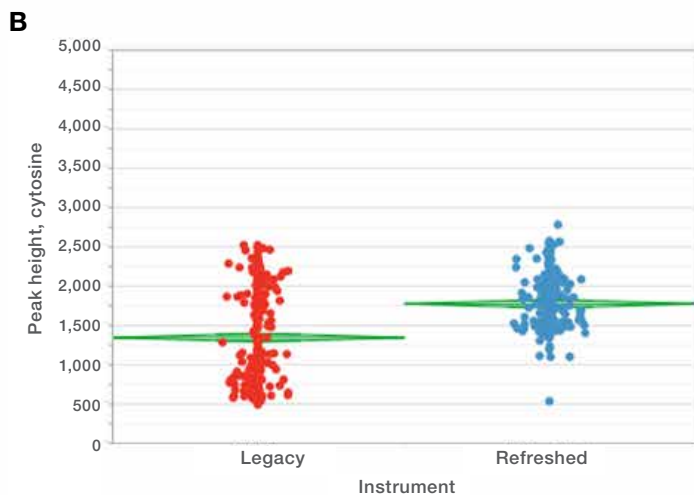


Instrument	Mean	SD
Legacy	1,281.04	313.00
Refreshed	1,599.01	250.07

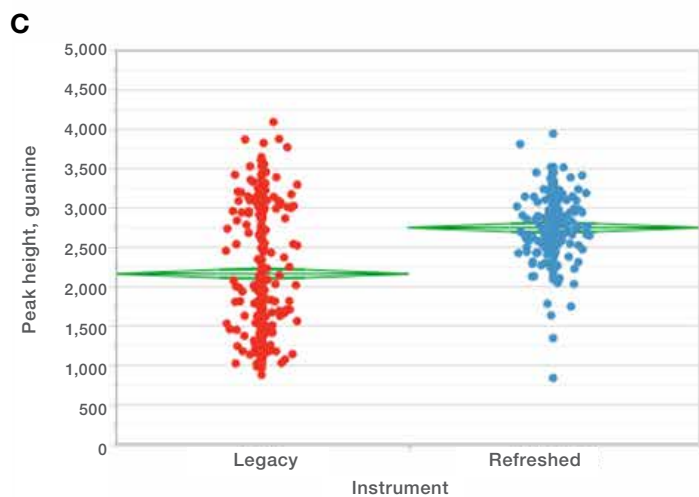
**Figure 6. One-way ANOVA of peak heights of sequencing analysis dye signals for the 48-capillary array, 36 cm configuration. Data are shown for (A) adenine, (B) cytosine, (C) guanine, and (D) thymine.**



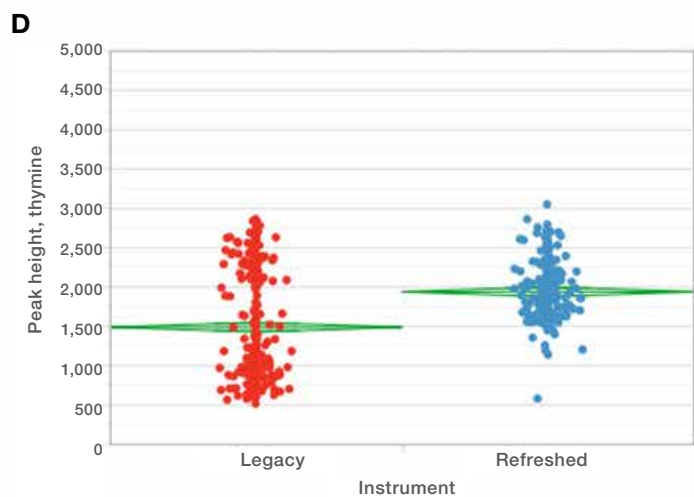
Instrument	Mean	SD
Legacy	2,032.69	948.50
Refreshed	2,751.53	428.26



Instrument	Mean	SD
Legacy	1,345.95	600.81
Refreshed	1,775.41	303.14



Instrument	Mean	SD
Legacy	2,163.79	813.31
Refreshed	2,750.20	360.97



Instrument	Mean	SD
Legacy	1,490.54	692.64
Refreshed	1,940.19	345.48

**Figure 7. One-way ANOVA of peak heights of sequencing analysis dye signals for the 96-capillary array, 50 cm configuration.** Data are shown for (A) adenine, (B) cytosine, (C) guanine, and (D) thymine.

## Conclusions

Sequencing data were collected with three legacy and three refreshed 3730 Series DNA Analyzers. Fragment analysis data were collected with two legacy and eight refreshed 3730 Series DNA Analyzers. Legacy and refreshed instruments perform similarly in sequencing and fragment analysis, generating comparable data. Q20 CRL, sizing precision, and sizing accuracy requirements are met with refreshed instruments and overall performance is similar to legacy instruments. New and existing customers can expect the same reproducible, high-quality results with the latest 3730xl DNA Analyzers, maintaining the same robust performance as the previous generation of instruments.

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