APPLICATION NOTE

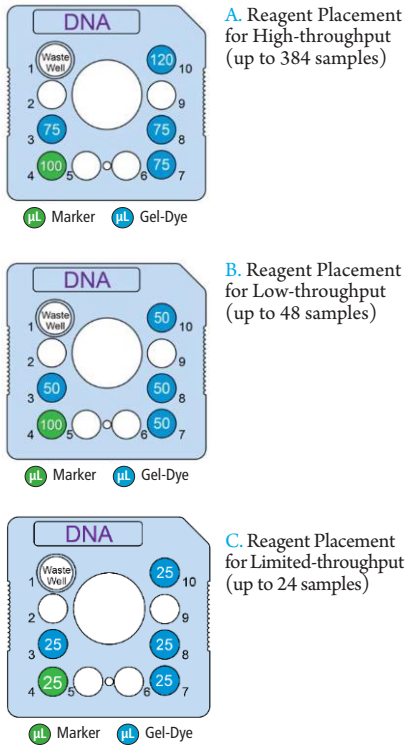
Nucleic Acid Analysis

LabChip® GX Touch™ Nucleic Acid Analyzer

Cost-effective Solution for Analyzing 24 samples or Less on the LabChip® GX Touch™ Nucleic Acid Analyzer

Introduction

Accurate qualitative and quantitative analysis of nucleic acids is a critical step prior to performing downstream processes. The LabChip® GX Touch™ nucleic acid analyzer offers both low-throughput analysis of up to 48 samples (Figure 1B) and for those with larger sample numbers, high-throughput analysis (Figure 1A) of up to 384 samples in a single run. However, this workflow can still exceed customer needs where only a few samples are analyzed. To that end, we have developed a limited-throughput protocol for nucleic acid analysis with reduced reagent volume to maintain similar overall cost per sample analysis. Herein, we evaluate the accuracy and consistency of the LabChip GX Touch nucleic acid analyzer using the limited-throughput protocol as compared to the higher-throughput protocols. The validation study demonstrates that up to 24 samples can be analyzed with the limited-throughput protocol and still maintain assay specifications for the LabChip GX DNA High Sensitivity Assay.



A. Reagent Placement for High-throughput (up to 384 samples)

B. Reagent Placement for Low-throughput (up to 48 samples)

C. Reagent Placement for Limited-throughput (up to 24 samples)

Figure 1. Comparison of reagent volume placement during chip preparation in high-throughput, low-throughput, and limited-throughput protocols.

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Results & Discussion

To validate the limited-throughput chip preparation protocol, we analyzed two sample sets: DNA fragments for molecular sizing and concentration and NGS library smears for size distribution and quantitation. The data concluded that assay performance was not affected by the reduction in reagent volumes loaded onto the chip; the assay adheres to the published specifications.

DNA Fragment Analysis. Table 1 summarizes the results from the measurements of DNA fragment mixtures tested with the limited-throughput protocol. Samples with a concentration of 0.019 ng/µL were chosen as an example. Sizing of 100 bp, 500 bp, and 1500 bp fragments were very accurate with less than 5% deviation and less than 2% sizing CV. Additionally, the concentration measurements for these fragments were consistent with those measured using a Qubit® fluorometer (Thermo Fisher Scientific®), with less than 10% concentration CVs observed.

Table 1. Summary of fragment sizing and concentration measurements

	Average Length (bp)	Concentration Measured with Qubit® Fluorometer, Thermo Fisher® (ng/uL)	Concentration Measured with LabChip® GX Touch™ Nucleic Acid Analyzer (ng/uL)	Concentration CV	Sizing Accuracy	Sizing CV
100 bp	101	0.019	0.0179	5.90%	1.02%	1.08%
500 bp	498	0.019	0.0156	7.98%	0.49%	0.34%
1500 bp	1575	0.019	0.0164	7.30%	5.01%	1.50%

Smear Analysis. Libraries prepared using the Illumina® Nextera® rapid capture kit were diluted prior to validation (Table 2). For all four concentrations evaluated (N=3), the electropherograms were consistent with specifications provided in the Illumina® library reference guide. As shown in Table 2, the concentrations measured with the LabChip GX DNA High Sensitivity Assay for the limited-throughput (i.e. low-volume) protocol were highly correlated to orthogonal measurements with a Qubit® fluorometer (Thermo Fisher Scientific®). The CV for samples at 5 ng/µL, 1 ng/µL, and 0.5 ng/µL, and 01 ng/µL were less than 2%.

Table 2. Summary of smear concentration measurements

Concentration Measured with Qubit® Fluorometer, Thermo Fisher Scientific® (ng/uL)	Concentration Measured with LabChip® GX Touch™ Nucleic Acid Analyzer (ng/uL)	Concentration Precision
5.0	3.86	0.82%
1.0	1.02	0.80%
0.5	0.52	1.41%
0.1	0.09	1.14%

Conclusion

In this study, we have demonstrated that when running less than 24 samples, the limited throughput chip preparation protocol does not affect assay performance. Furthermore, the data shows precise concentration and molecular weight sizing measurements. The limited-throughput chip preparation protocol reduces the cost per sample providing a cost effective workflow to analyze low sample numbers.

It should be noted that when running more than 24 samples, marker well regent depletion is observed. Similarly, when running less than 24 samples over an eight hour period, we suggest the high-throughput (Figure 1B) chip preparation protocol due to evaporation over time.

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