PrepQuant™ System: Integrates Nucleic Acid Extraction, Concentration, and Quantification into a Single Automated Workflow

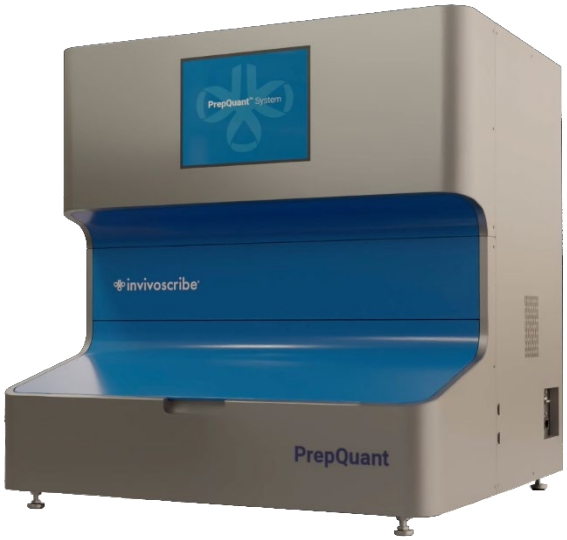
INTRODUCTION

Sample preparation remains a significant source of variability in downstream molecular testing workflows. Nucleic acid extractions often require multiple laboratory instruments to perform extraction, concentration, and quantification, increasing hands-on time and introducing opportunities for variability. Automated systems can reduce this complexity and improve workflow consistency. PrepQuant, integrates nucleic acid extraction, concentration, and quantification into a single automated system.

Here we present the performance of the PrepQuant System for peripheral blood (PB) and bone marrow (BM) specimens processed using the PrepQuant™ Genomic DNA EQ 400 and PrepQuant™ Genomic DNA ECQ 2000 workflows. Extracted genomic DNA (gDNA) was evaluated for concentration, purity, and correlation with Qubit 3.0 as the reference quantification method. Downstream molecular assay compatibility was evaluated using LeukoStrat® *FLT3* ITD MRD Assay on the Illumina MiSeq™ Dx.

PrepQuant System Overview

The PrepQuant System (**Figure 1**) uses single-use cartridges that contain the reagents required for automated gDNA extraction and onboard quantification. After sample is loaded into the designated cartridge well, magnetic bead-based chemistry is used to lyse sample, bind DNA, remove impurities through wash steps, and elute purified DNA. For workflows that include a concentration step, DNA is concentrated automatically during processing. The concentration of eluted DNA is then measured onboard using an integrated fluorometer and cartridge-contained quantification standards. For gDNA extraction workflows, the sample input volume is 400 µL for the PrepQuant Genomic DNA EQ 400 kit and 2000 µL for the PrepQuant™ Genomic DNA ECQ 2000 kit.



Workflow

To perform a nucleic acid extraction run on the PrepQuant System, a peripheral blood (PB) or bone marrow (BM) sample is loaded into the designated well of a single-use reagent cartridge. The cartridge, together with disposable pipette tips and an output tube, is placed into the instrument. After the appropriate workflow is selected, the system automatically performs DNA extraction, optional concentration depending on workflow, and onboard quantification. The PrepQuant System supports up to 24 samples per run.

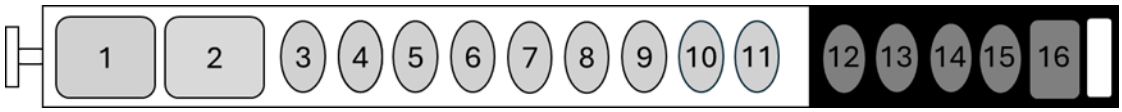


Figure 1. Reagent Cartridge. Sample is added to Well #1 for Genomic DNA ECQ 2000 or Well #3 for Genomic EQ 400 Kits.

MATERIALS AND METHODS

PrepQuant Study Sample Set

Two instrument performance studies were conducted: one for the EQ 400 workflow and one for the ECQ 2000 workflow. Each study included 24 specimens, comprising of 12 peripheral blood (PB) and 12 bone marrow (BM) specimens. Positive-control specimens were included, as applicable, using internal IVS-0070 spike-in positive-control DNA in negative PB or BM matrices. In the EQ 400 study, the positive PB set consisted of disease-positive clinical specimens, and the positive BM set included disease-positive clinical specimens together with one spike-in positive BM specimen. In the ECQ 2000 study, the positive PB set comprised spike-in positive PB specimens, and the positive BM set comprised both disease-positive clinical specimens and spike-in positive BM specimens. When pre-extraction cell counts were not available, specimens were measured prior to extraction using the Auto2000 Cellometer™. Specimens below the nominal ≥5.0 × 10^6 TNC/mL threshold were still processed and documented in the study reports. In the EQ 400 study, 5 of 24 specimens were below this threshold; in the ECQ 2000 study, 7 of 24 specimens were below this threshold.

PrepQuant Operation

For both workflows, specimens were loaded into PrepQuant reagent cartridges and processed according to the corresponding PrepQuant workflow instructions. The EQ 400 workflow used 400 µL input volume per specimen, and the ECQ 2000 workflow used 2000 µL input volume per specimen. Following cartridge and consumable loading, the selected workflow was initiated on the PrepQuant System, which automatically performed extraction, concentration as applicable, and onboard quantification.

Reference Quantification and Purity Assessment

PrepQuant concentration results were generated from the onboard GelGreen®-based fluorescence readout. Reference DNA quantification was performed using Qubit 3.0, and sample purity was assessed using NanoDrop™ 2000 by A260/A280 ratio. Comparator extractions were performed using the most comparable manufacturer-recommended workflows for KingFisher™ Flex and EZ2™ Connect, as applicable.

Table 1. Study Specimen Panel

Workflow	Specimen Type	Positive/Target-Positive Specimens	Negative Specimens
EQ 400	Peripheral Blood	6	6
EQ 400	Bone Marrow	6*	6
ECQ 2000	Peripheral Blood	6*	6
ECQ 2000	Bone Marrow	6*	6

*Includes donor-positive and/or IVS-0070 spike-in positive specimens, as applicable to the workflow.

FLT3 ITD MRD Sequencing

To evaluate downstream molecular assay compatibility, PrepQuant extracted DNA was amplified, prepared, and sequenced according to the LeukoStrat® *FLT3* ITD MRD Assay instructions for use on the Illumina MiSeq Dx using the MiSeq Reagent Kit v3 (600-cycle) and paired-end 2 × 300 bp sequencing. Sequencing run validity was assessed using run summary outputs, including Q30 and assay control performance, and sequencing data were analyzed using the *FLT3* ITD MRD software specified in the assay workflow. Samples used for this analysis had been previously characterized by LabPMM®, a CAP/CLIA certified laboratory in San Diego CA, and results from PrepQuant extracted samples were compared with those prior results. Across the three MiSeq runs used in this study (M7F6L, M7ZGF, and M6P9M), all runs met run-validity criteria. Reported Q30 values were 78.78%, 85.17%, and 87.5%, respectively, and positive, negative, and no-template controls passed in all runs. Detailed run metrics are summarized in **Table 2**.

Table 2. *FLT3* MRD Sequencing Run Metrics

Metric (per Flow Cell)	Run 1	Run 2	Run 3
Run/Flowcell ID	M7F6L	M7ZGF	M6P9M
Loading Concentration(pM)	14	14	14
Average % ≥ Q30	78.78%	85.17%	87.5%
% Clusters Pass Filter	93.14%	96.93%	95.73%
Total Output (Gb)	12.95	12.93	12.93
Cluster Density (k.mm^2)	949.01 +/- 16.1	879.16 +/- 24.79	925.62 +/- 16.01
Run Validity	Valid	Valid	Valid

RESULTS

PrepQuant System generated high-purity DNA across both workflows

PrepQuant met the predefined purity acceptance criterion in both workflow studies. In the EQ 400 study, 24 of 24 samples (100%) achieved a 260/280 ratio ≥1.8 (**Table 3**). In the ECQ 2000 study, 24 of 24 samples (100%) also achieved a 260/280 ratio ≥1.8. For the EQ 400 comparator dataset, PrepQuant showed a higher proportion of samples meeting the purity threshold than either comparator platform, with 24/24 (100%) for PrepQuant, 16/24 (67%) for KingFisher Flex, and 8/12 (67%) for EZ2 Connect. These results support emphasizing both purity and output concentration range, rather than purity alone.

Table 3. DNA Concentration Range and Purity by Workflow

Kit	Input Volume (µL)	Specimen Type	Concentration Range (ng/µL)	Purity Range (260/280)
EQ 400	400	Peripheral Blood	8.5-292.9	1.80-1.88
EQ 400	400	Bone Marrow	30.5-482.8	1.79-1.88
ECQ 2000	2000	Peripheral Blood	544.7-1076.9	1.82-1.90
ECQ 2000	2000	Bone Marrow	144.6-1353.3	1.80-1.95

PrepQuant quantification compared with the reference quantification method

PrepQuant onboard quantification correlated strongly with Qubit 3.0 in both workflows (**Figure 3** and **Figure 4**). For the EQ 400 workflow, the correlation coefficient was R^2 = 0.991 across peripheral blood and bone marrow specimens. For the ECQ 2000 workflow, the correlation coefficient was R^2 = 0.902 across peripheral blood and bone marrow specimens. These data support the use of the PrepQuant onboard quantification output as a reliable indicator of sample concentration across both workflows.

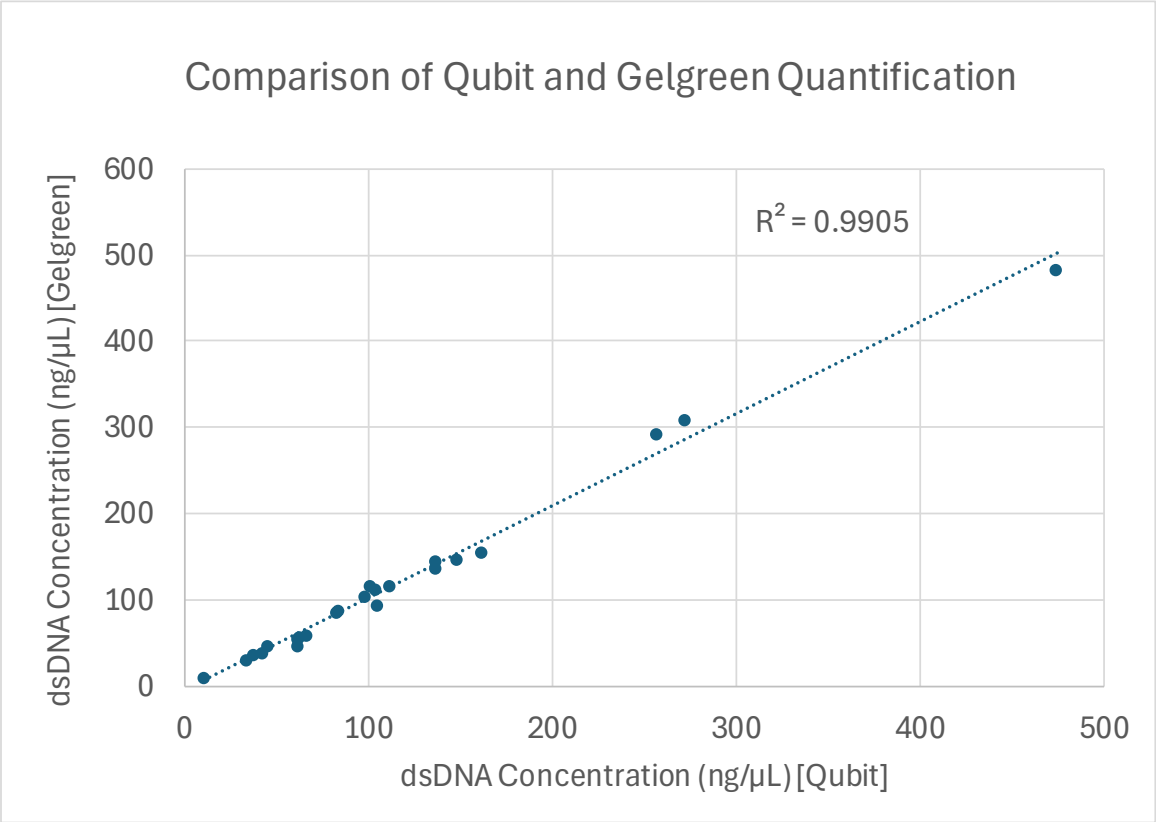


Figure 3. EQ 400 PrepQuant onboard quantification versus Qubit 3.0 reference quantification

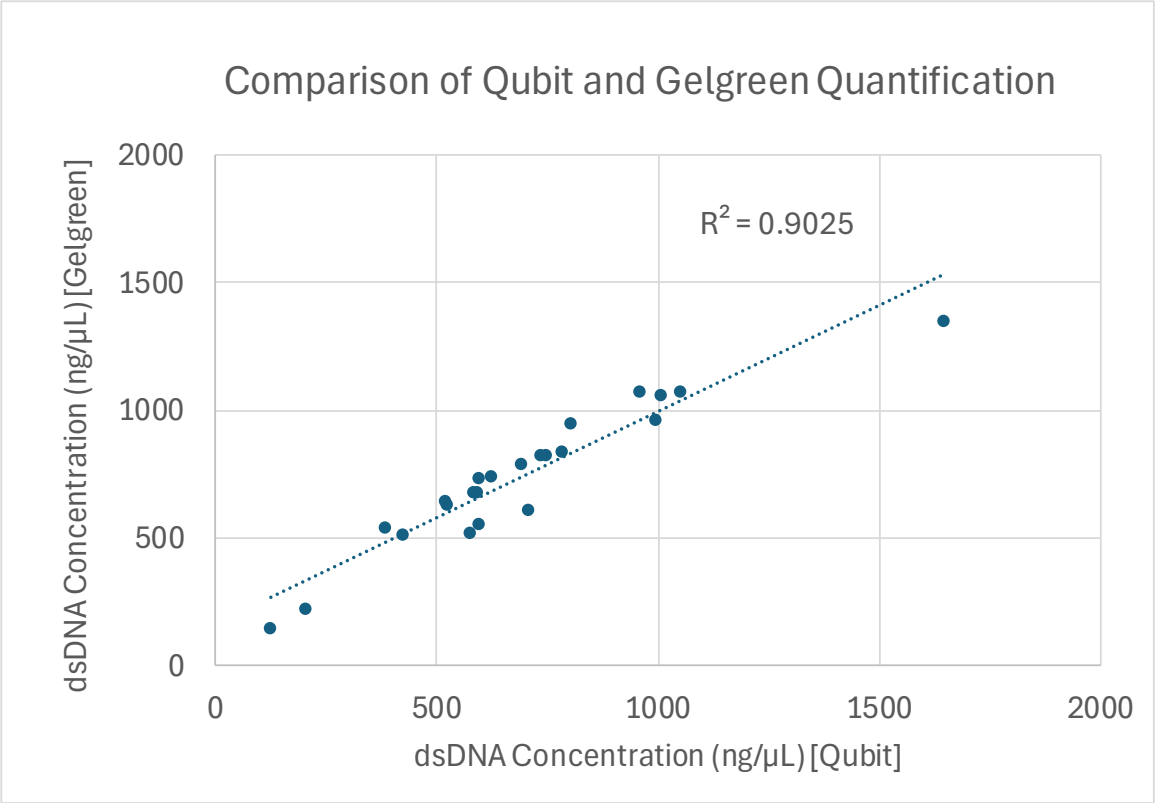


Figure 4. ECQ 2000 PrepQuant onboard quantification versus Qubit 3.0 reference quantification

PrepQuant DNA extracts performance on *FLT3* ITD MRD Assay

All three MiSeq Dx sequencing runs passed assay run-validity criteria, and all run controls passed. These data support the compatibility of PrepQuant extracted DNA with the LeukoStrat *FLT3* ITD MRD Assay workflow on the Illumina MiSeq Dx. Among evaluable results, concordance with prior LabPMM characterization was 6/6 (100.0%) for positive PB samples, 4/4 (100.0%) for negative PB samples, 4/4 (100.0%) for positive BM samples, and 5/5 (100.0%) for negative BM samples (**Table 4**). Samples with undetermined sequencing results were excluded from concordance analysis.

Table 4. Concordance with LabPMM *FLT3* ITD MRD Sequencing Results

Specimen Type	LeukoStrat *FLT3* ITD MRD Result	Concordant PrepQuant Results
Peripheral Blood	Positive	100.0% (6/6)
	Negative	100.0% (4/4)
Bone Marrow	Positive	100.0% (4/4)
	Negative	100.0% (5/5)

CONCLUSION

In summary, the PrepQuant System generated high-purity DNA across both extraction workflows, produced broad and application-relevant gDNA concentration ranges, and showed strong agreement between onboard quantification and Qubit 3.0 reference quantification. PrepQuant extracted DNA was compatible with downstream testing using the LeukoStrat *FLT3* ITD MRD Assay on the Illumina MiSeq Dx, with all sequencing runs meeting run-validity criteria. Together, these data support PrepQuant as an integrated workflow solution for automated genomic DNA extraction, concentration, and onboard quantification.

ABOUT INVIVOSCRIBE

Invivoscribe is a global, vertically integrated biotechnology company dedicated to Improving Lives with Precision Diagnostics®. For over thirty years, Invivoscribe has enhanced the quality of healthcare worldwide by providing high-quality, standardized reagents, tests, and bioinformatics tools to advance the field of precision medicine. With the launch of the PrepQuant System, the Invivoscribe ecosystem now includes instrumentation, extending our standardization efforts beyond diagnostic tests to include the critical pre-analytical steps that ensure accuracy and reproducibility from start to finish.

PrepQuant System and Reagent Cartridges

Catalog #	Product Description	Quantity
P100100	PrepQuant™ System	1 each
1100001	Genomic DNA ECQ 2000 Kit	48 preps
1100002	Genomic DNA EQ 400 Kit	48 preps

To learn more about how the PrepQuant System can standardize the pre-analytical workflow for your laboratory, please email **inquiry@invivoscribe.com**. To learn more about Invivoscribe and LabPMM, visit us online at **invivoscribe.com** or call us at **+1.858.224.6600**.