Application Note

A front-end automation method for QIAcuity digital PCR plate setup using the QIAgility liquid handling instrument

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Introduction

Digital PCR is an absolute quantification method that is growing in popularity as a superior method to qPCR-based technologies. The QIAcuity Digital PCR System delivers precise and multiplexed quantification results for mutation detection, copy number variation (CNV), gene expression studies, gene-editing analysis, and many more applications, including those used in high-throughput settings.

Accurate and consistent pipetting is critical to ensure that results are reproducible across operators, experiments, and even within the same plate. Setting up PCR reactions for any PCR-based platform is susceptible to human error, resulting in contamination, inaccurate concentration determination, high variability across replicates, false positives, and/or false negatives. To overcome potential pipetting mistakes in setting up dPCR plates for QIAcuity analysis, we developed a method for the front-end automation of dPCR nanoplate setup using the QIAgility liquid handling instrument. The QIAgility instrument is a liquid handler designed for automating PCR setup. For compatibility with the QIAcuity, we developed an adapter (Figure 1) to secure up to two nanoplates onto the deck of the QIAgility. Using the QIAgility software, we have optimized a protocol that works for all nanoplate types and QIAcuity applications. Here we report the performance of a front end automated QIAgility dPCR nanoplate setup procedure for use with the QIAcuity dPCR system.

Figure 1. QIAgility adapter for dPCR nanoplates.

Methods

A QIAcuity master mix containing all required components for an ERBB2 copy number assay was first prepared manually. This master mix was used subsequently for all nanoplates, whether prepared manually or via the QIAgility. A single sample known to have an elevated ERBB2 copy number was used for setting up all the nanoplates for this study (Figure 2 A).

Manual nanoplate setup

Three dPCR nanoplates were set up manually using the master mix and sample described above (Figure 2 A). For each plate, the sample and master mix were added to each well of a 96-well PCR plate, thoroughly mixed, ▶

and then transferred to a 96-well dPCR nanoplate, as recommended (Figure 2 B). This setup procedure consisted of 288 individual pipetting steps per plate (96 additions of master mix, 96 additions of sample, 96 transfers to nanoplate), resulting in 864 pipetting steps for the setup of the three nanoplates.

QIAgility nanoplate setup

Three dPCR nanoplates were set up using the QIAgility liquid handler. Briefly, the QIAgility adapter for nanoplates (Figure 1) was secured onto the deck of the QIAgility, and the nanoplate was placed within the adapter. The QIAgility was then calibrated to the dimension of the nanoplate. The ERBB2 master mix and sample were used as described above. For each plate, the master mix and sample were added directly to the nanoplate, thus saving time and plasticware (Figure 2 C). Once the protocol was executed, it took the QIAgility 35 minutes to set up each 96-well nanoplate. No manually pipetting steps were required.

Analysis

Each resulting nanoplate was analyzed on a QIAcuity instrument (Figure 2D). Sample loading, average number of valid partitions, and average concentration values for ERBB2 were analyzed and compared.

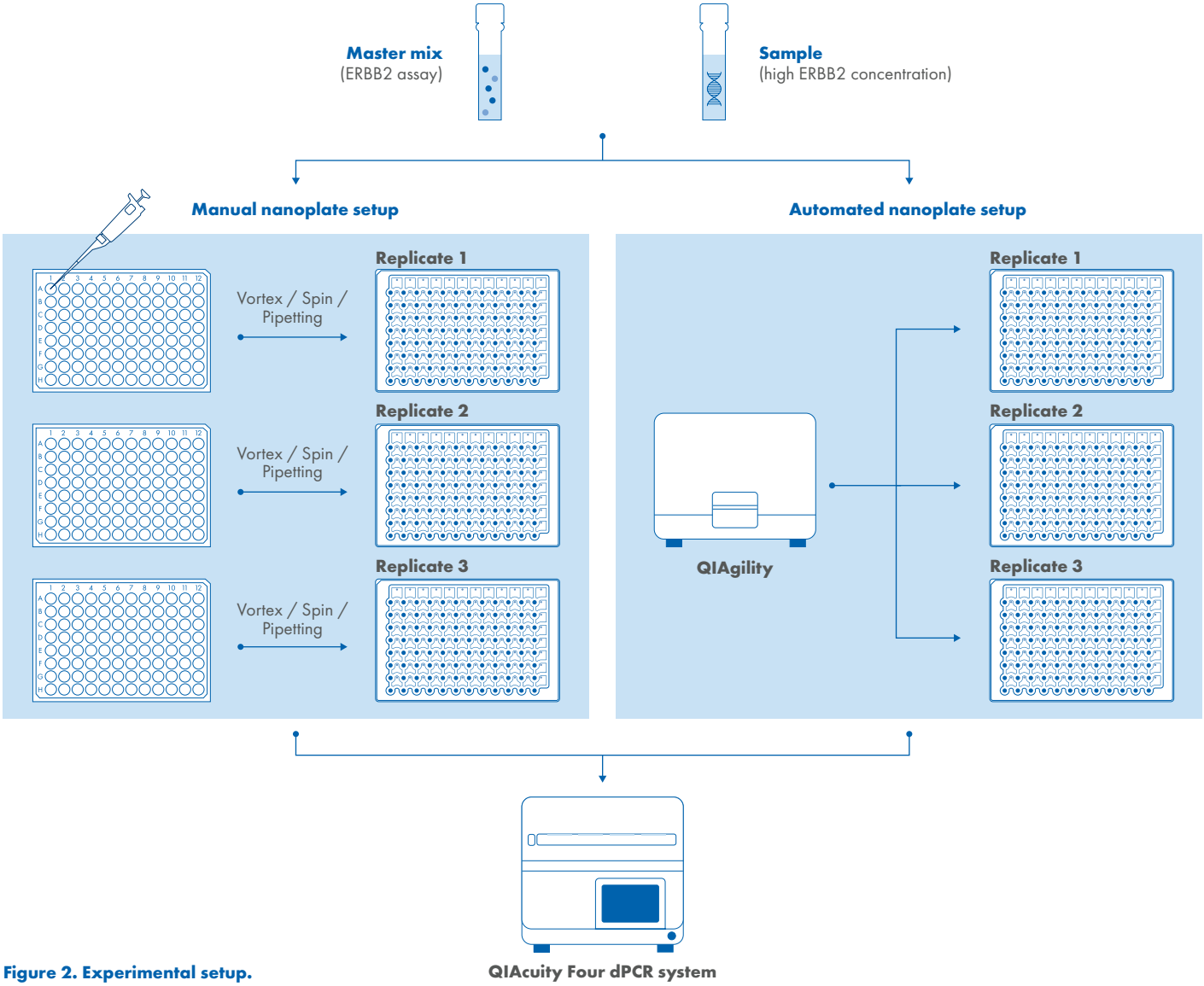
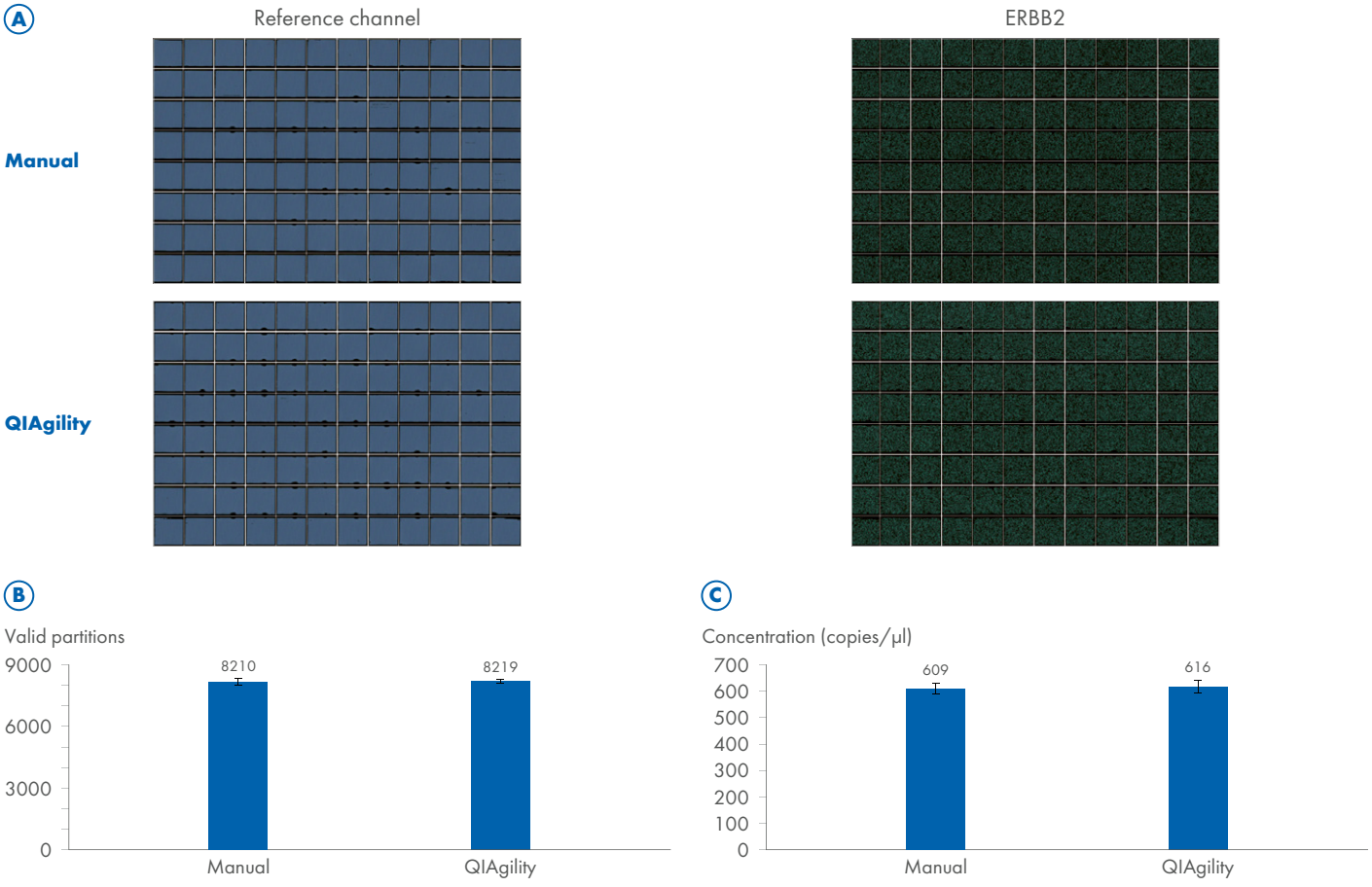


Figure 2. Experimental setup.

Results

To assess the performance of the QIAgility system in setting up dPCR nanoplates for QIAcuity dPCR analysis, we set up three different 96-well nanoplates manually and three additional 96-well nanoplates via the automated QIAgility protocol (Figure 2). The six resulting nanoplates were analyzed on a QIAcuity instrument.

Even sample loading was observed for all six plates, regardless of the setup method (Figure 3 A). Across the six nanoplates analyzed, the number of partitions for each plate was within error (%CV = 1-2%) of one another, demonstrating that both methods result in equivalent, if not identical, numbers of valid partitions (Figure 3 B). Further demonstrating this near-identical performance across the two methods, the average concentration for ERBB2 was 616 ± 25 and 609 ± 25 copies/µl, respectively, and thus were within measurement error of one another (Figure 3 C). Furthermore, the variation (%CV) across valid partition numbers for each plate (Figure 3 B) and concentration (Figure 3 C) were identical for both methods.



	Manual	QIAgility
Valid partitions	8210	8219
CV%	1.9%	1.0%

	Manual	QIAgility
Concentration (copies/µl)	609	616
CV%	3.5%	3.8%

Figure 3. Consistent sample loading, valid partitions, and concentrations were observed across both the manual and the QIAgility automated methods.
Three nanoplates were manually prepared and compared with three nanoplates prepared with the QIAgility instrument. Representative samples are shown. **A** Images of each well show even sample loading using the reference channel and the ERBB2. **B** The average number of valid partitions was calculated over the three plates for the manual and QIAgility protocols. **C** The average concentration values for ERB22 concentration were calculated for each set of plates.

Experiments were replicated for the 24-well nanoplates, and no significant differences in performance between the manual and automated methods were observed. For brevity, the performance of the QIAgility in setting up 24-well nanoplates is provided in summary only (Table 2).

To assess inter-instrument reproducibility, an additional three plates were set up with the same parameters described above but were run on a second QIAgility instrument. The number of valid partitions and variation across replicates (%CV) were analyzed and compared. The number of valid partitions as well as the concentrations measured for the six plates set up on the two different instruments were within error of one another, demonstrating a low level of inter-instrument variability (Table 1).

Table 1. Consistent performance was observed across two separate QIAgility instruments

		QIAgility Instrument 1	QIAgility Instrument 2
Valid Partitions	Measured	8,214	8,226
	Variation across replicates (% CV)	1.8%	1.5%
Concentration	Measured (copies /µl)	924	918
	Variation across replicates (% CV)	3.5%	3.8%

To assess the contamination risk of using the QIAgility for dPCR nanoplate setup, two master mixes containing different dyes were added using vertical and horizontal zebra layouts before internal control samples were pipetted to every well of a 96-well nanoplate (Figure 4). The resulting plates were analyzed by the QIAcuity dPCR system using the green and red channels to detect the respective dye of the master mix. No detection or 'spillover' was observed in wells that show no positive partitions, demonstrating a low risk for QIAgility produced contamination events.

In total, we analyzed three different 96-well nanoplates prepared manually, resulting in a total of 288 wells, of which 286 (99.3%) were usable. Using the QIAgility, a total of 15 different 96-well nanoplates were prepared, resulting in a total of 1,440 wells of which 1,428 (99.1%) were usable. The results in Table 2 show 1) variation across replicates (%CV), 2) variation across instruments (%CV), and 3) the risk of spillover contamination. The same experiments were performed using 26k 24-well nanoplates which, like the 8.5k, 96-well nanoplates, also demonstrated equivalent performance to the manual method (Table 2). In summary, across all wells analyzed, near-identical valid partition numbers, concentrations, and plate loading performance were observed for both manually prepared and QIAgility prepared dPCR nanoplates.

Figure 4. No spillover contamination events were observed using the QIAgility for dPCR nanoplate setup.
Two master mixes were pipetted using vertical and horizontal zebra layouts before the sample was added to every well. The results shown are positive partitions measured via fluorescence in either the **A** Green Channel or the **B** Red Channel for each well within a single 96-well nanoplate. One well could not be analyzed due to standard filling errors, which are occasionally observed on the QIAcuity using plates prepared both manually and via the QIAgility.

Table 2. Equivalent performance was observed across different methods and instruments

	Nanoplate 26k, 24-well				Nanoplate 8.5k, 96-well			
	Method comparison		Inter-instrument comparison		Method comparison		Inter-instrument comparison	
	Manual	QIAgility	QIAgility Instrument 1	QIAgility Instrument 2	Manual	QIAgility	QIAgility Instrument 1	QIAgility Instrument 2
Variation across replicates – Total Valid Partitions (%CV)	4.3%	0.6%	1.2%	3.1%	1.9%	1.0%	1.8%	1.5%
Variation across replicates – Concentration (%CV)	7.4%	1.4%	1.2%	1.0%	3.5%	3.8%	2.9%	3.7%
Contaminated wells (%)	0%	0%	–	–	0%	0%	–	–

Conclusions

Digital PCR is being used more routinely by an increasing number of labs for an expanding range of applications. Due to growing interest in front-end automation, an adapter to fit dPCR nanoplates to the QIAgility liquid handler has been developed (Figure 1). We report herein that setting up dPCR nanoplates on the QIAgility produces comparable, or even improved, reproducibility over a manual setup method. The main benefit of using the QIAgility for front-end automation setup of dPCR nanoplates is that it eliminates human errors, such as variation and contamination. Additionally, automation saves operator time, allowing up to two nanoplates to be set up in one QIAgility protocol, which is particularly important in high-throughput environments.

Ordering Information

Product	Contents	Cat. no.
QIAcuity Probe PCR Kit	1 ml 4x concentrated QIAcuity Probe Master Mix, 2 x 1.9 ml Water	250101
QIAcuity Nanoplate 8.5k 96 well	10 QIAcuity Nanoplates 8.5k 96-well, 11 Nanoplate Seals	250021
QIAcuity Nanoplate 26k 24 well	10 QIAcuity Nanoplates 26x 24-well, 11 Nanoplate Seals	250001
QIAcuity Nanoplate Adapter	For holding 1x QIAcuity Nanoplate; for use with the QIAgility to automate nanoplate set up before digital PCR reaction on the QIAcuity Digital PCR System	9027242
QIAgility System	Instrument and service agreement package: robotic workstation for automated PCR setup (with UV light and HEPA filter), notebook computer and QIAgility software; includes installation, application training and one-year warranty on labor, travel and parts.	9001532
QIAcuity Four Platform System	Four-plate digital PCR instrument for detecting up to 5 fluorescent dyes, notebook computer, barcode scanner, roller, USB flash memory and QIAcuity Software Suite: includes installation, training, and 1 preventive maintenance visit, 1 year warranty on labor, travel, and parts	911042

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For more information, visit **www.qiagen.com/dPCR**.