

Plug-and-play digital PCR: Automated QIAcuity® Nanoplate setup on Eppendorf epMotion®

Abstract

Automating QIAcuity® Nanoplate setup on the epMotion® streamlines digital PCR (dPCR) sample preparation by reducing workflow steps, tips and PCR plate consumption, as well as hands-on time by up to 50% compared to manual pipetting. The automated workflow reduced operator-dependent variability associated with manual pipetting and generated results comparable to manual preparation under the evaluated

conditions. Herein, we demonstrate that the compact and open epMotion 5073 platform enables automated dPCR setup of QIAcuity Nanoplates across multiple formats (26k 24-well and 8.5k 96-well), delivering reliable performance while increasing walk-away time for users. Moreover, epMotion 5073 can be readily repurposed for additional applications such as sample dilution, normalization, and purification.

Key words

- Digital PCR (dPCR)
- QIAcuity® Nanoplate
- epMotion
- Automation
- Liquid handling

Introduction

Digital PCR (dPCR) delivers high sensitivity, absolute quantification, and robustness for a broad range of applications, from copy number variation to rare mutation detection [1]. However, manual preparation can be tedious, time-consuming, and operator-dependent, particularly in laboratories facing increasing sample numbers, potentially leading to variable outcomes. As dPCR enables detection of very low target quantities, the need for accurate, precise, and reproducible pipetting to ensure reliable data is further emphasized.

Automated liquid handling standardizes workflows, ensures accurate and reproducible liquid distribution, and frees up skilled staff for higher-value tasks. The Eppendorf epMotion platform is a flexible, open liquid handling system that can be adapted to multiple protocols while maintaining a small footprint.

Work recently done using the QIAGEN® QIAcuity® platform already showed that epMotion automation reliably performs dPCR Nanoplate setup with good performance while standardizing key pipetting steps [2].

In this work, we present an automated QIAcuity® Nanoplate setup on the epMotion in which samples and master mix are transferred directly into the Nanoplate, avoiding intermediate plates. This approach reduces the number of pipetting steps and consumption of tips and plates. It also shortens hands-on time while maintaining assay performance.

The epMotion 5073 is a particularly appealing option for laboratories seeking compact automation. It supports automated preparation of up to two Nanoplates per run and can be easily repurposed for additional applications beyond dPCR. For higher-throughput needs, the larger epMotion 5075 enables automated preparation of up to four QIAcuity Nanoplates 8.5K 96-well Nanoplates per run or up to eighth QIAcuity Nanoplates 24-well per run, offering a scalable pathway from small to medium-throughput dPCR automation.

In cooperation with QIAGEN® GmbH.



Materials and methods

Automated liquid handling was compared to manual liquid handling using one set of reagents and samples. The samples consisted of a mixture of genomic DNA (gDNA) and a small DNA fragment at a single concentration. dPCR was performed as a duplex assay, with one specific target sequence for each DNA type. The QIAcuity master mix (QIAcuity® Probe PCR Kit, QIAGEN® Cat No. 250101) containing the primers and the samples was prepared manually.

The dPCR setup was evaluated on QIAcuity® Nanoplate 8.5k 96-well (QIAGEN, Cat No. 250021) using 10 µL master mix and 2 µL sample, and on QIAcuity® Nanoplate 26k 24-well (QIAGEN, Cat No. 250001) using 36 µL master mix and 4 µL sample.

On epMotion 5073 (Eppendorf, Cat No. 5073000110), the dPCR master mix and the samples were directly distributed and mixed in the Nanoplate (Figure 1). The QIAcuity® Nanoplate adapter (QIAGEN, Cat No. 9027242) was utilised on the epMotion to securely hold the Nanoplate and enable reliable, homogeneous liquid distribution across all wells (Figure 2). The QIAcuity adapter was mounted onto an epMotion height adapter positioned on the deck to ensure its proper and secure fixation throughout the procedure.

In the manual process, the samples were mixed with the dPCR master mix in a reaction plate which is afterwards centrifuged. The reaction mixture was then subsequently added to the Nanoplate.

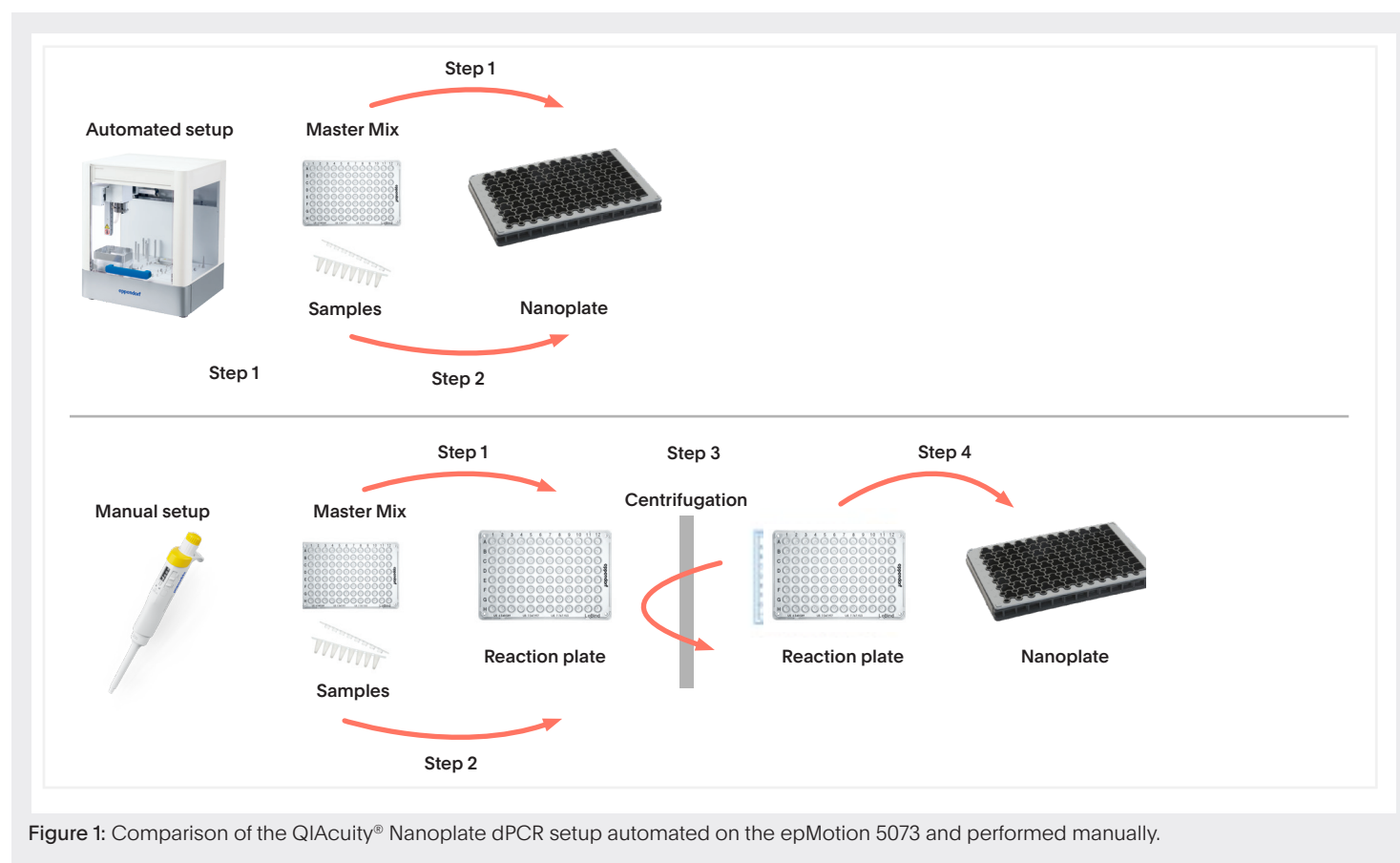
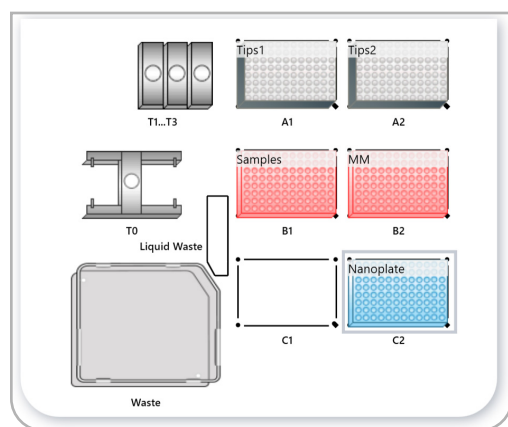


Figure 1: Comparison of the QIAcuity® Nanoplate dPCR setup automated on the epMotion 5073 and performed manually.



QIAcuity® Nanoplate Adapter

Figure 2: Deck layout representation of the dPCR setup for a QIAcuity® Nanoplate 8.5k 96 well on the epMotion 5073i. The Nanoplate is placed in a dedicated QIAcuity® Nanoplate Adapter on the epMotion deck.

Table 1: dPCR setup time and tip consumption to perform the dPCR setup for one Nanoplate. Manual versus automated on epMotion for different Nanoplate formats are shown. In both setups, the distribution of the master mix and the samples is performed using 8-channel dispensing tools

QIAcuity Nanoplate	dPCR setup time (minutes)		Time reduction epMotion vs. Manual	Tip consumption		Tip reduction epMotion vs. Manual
	epMotion	Manual		epMotion	Manual	
8.5k 96-well	12	15	20%	104	193	46%
26k 24-well	4	8	50%	32	49	35%

During the automated process, the direct addition of master mix and samples to the Nanoplate saves time, tips (Table 1) and additional plastic plate.

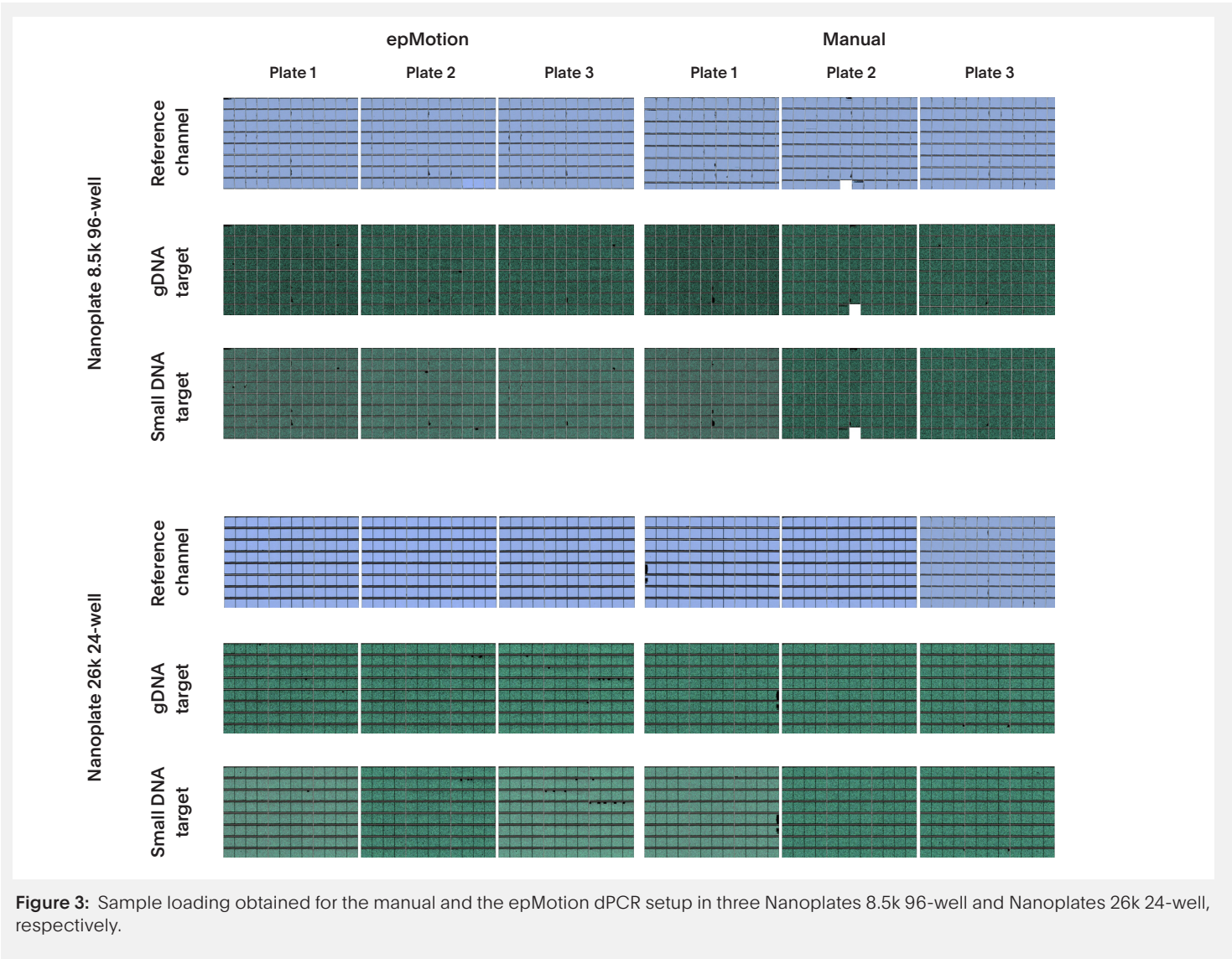
All dPCR runs were performed on a QIAcuity® One instrument from QIAGEN. The workflow described here is also applicable to QIAcuity® instruments with 4-plate and 8-plate capacity.

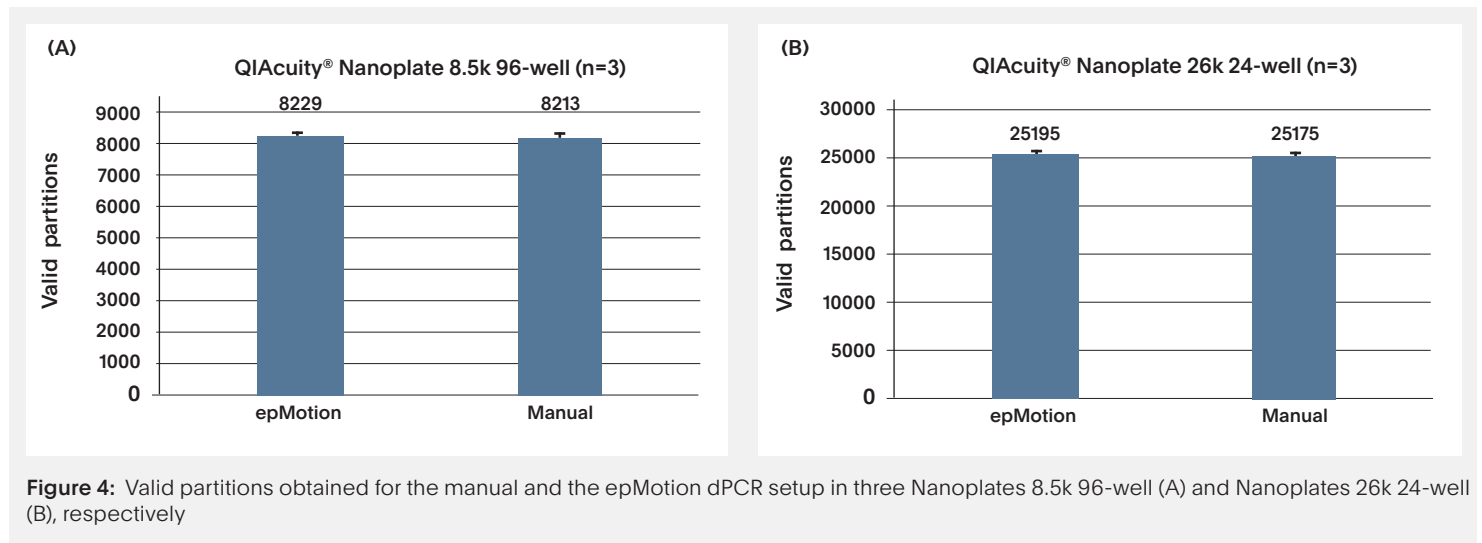
Sample loading uniformity, average number of partitions and average concentrations values were analysed using QIAcuity® Software Suite (SW Version 3.1.1.0).

Results

The performance of the QIAcuity dPCR setup was assessed on three Nanoplates 8.5k 96-well and 26k 24-well, respectively, prepared both manually and using the epMotion.

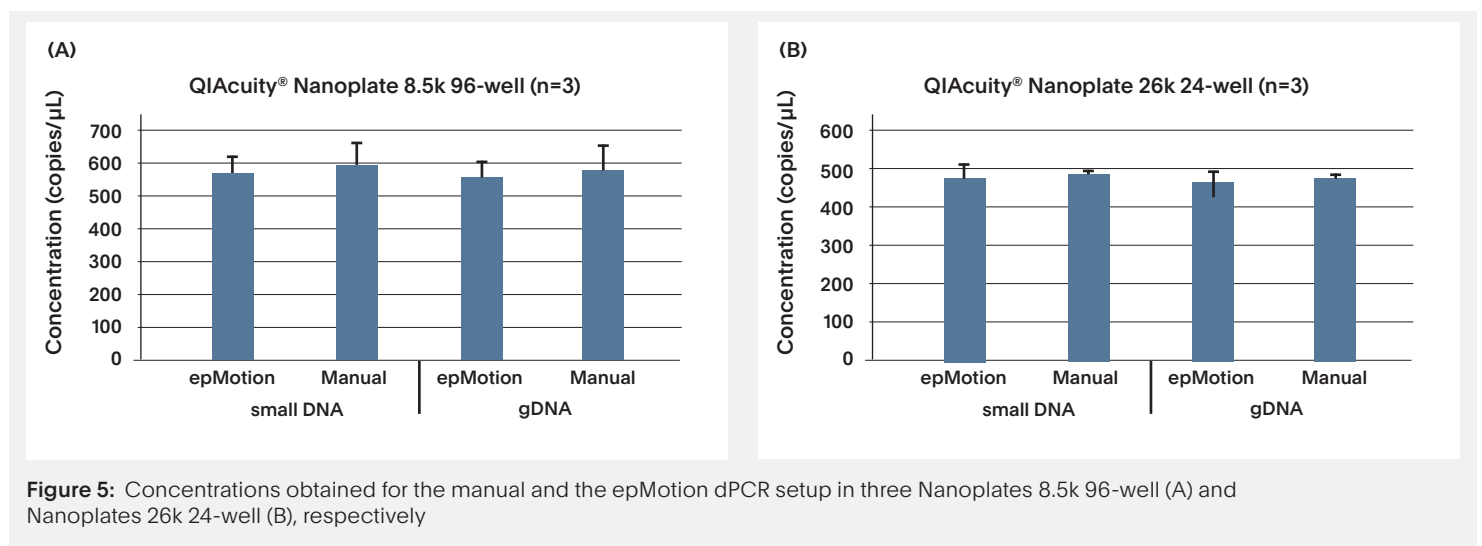
Uniform sample loading was observed for all Nanoplates, with overall comparable filling characteristics between automated and manual setups, although some variability was noted in the manually prepared plates (Figure 3).





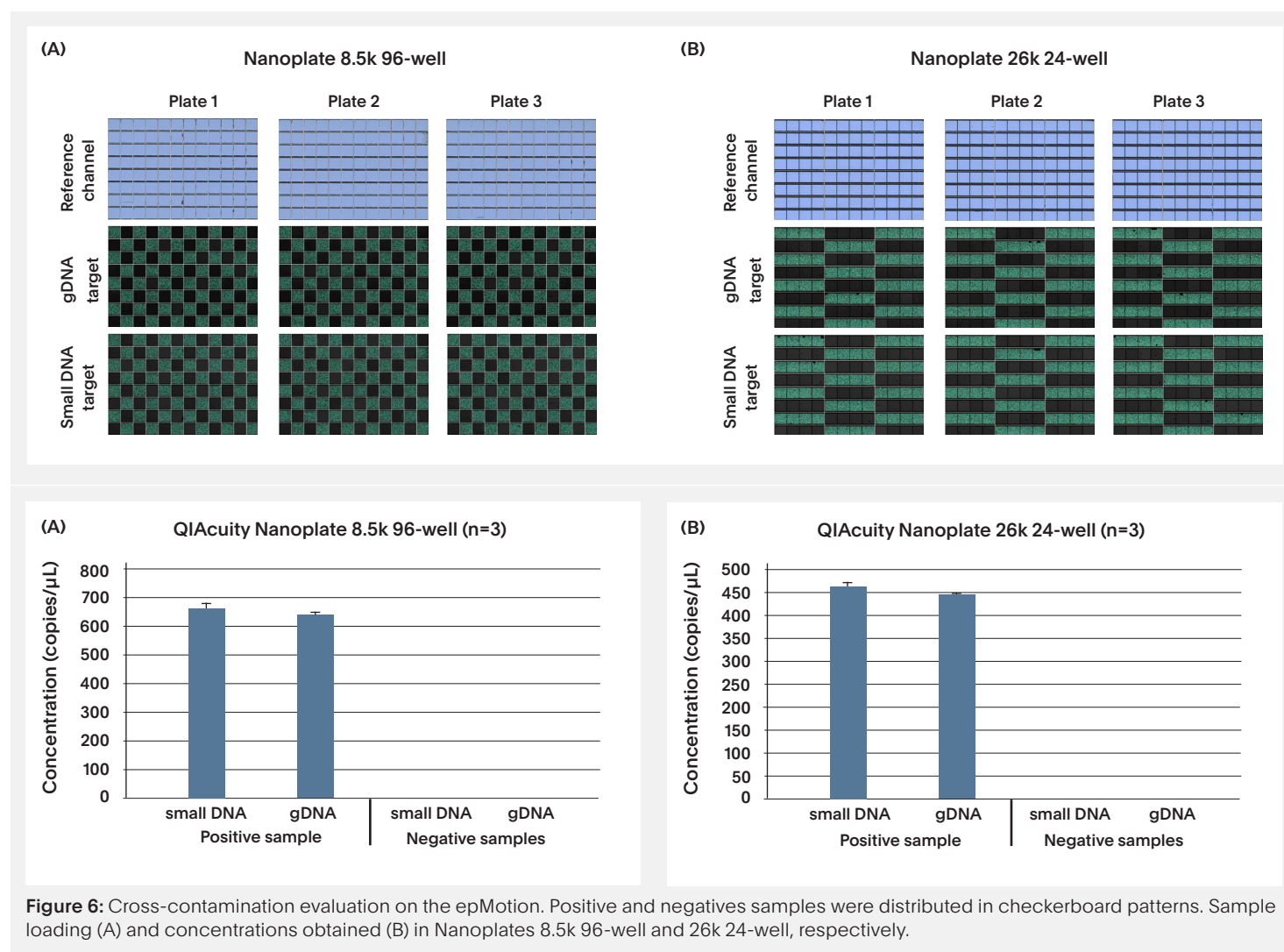
The number of partitions for plates prepared on the epMotion did not differ significantly from those prepared manually, regardless of the Nanoplate format used, demonstrating that both setup methods provide equivalent partition performance (Figure 4).

The two methods also yielded very similar results, as the average concentrations for both targets in small DNA and gDNA differed only within the expected measurement error (Figure 5).



To assess the cross-contamination risk of using the epMotion for dPCR Nanoplate setup, positive samples and negative controls (water) were added in a checkerboard pattern in three replicates per Nanoplate type.

No positive partitions were detected in the negative-control wells, indicating that the epMotion did not introduce cross-contamination during sample preparation (Figure 6).



In summary, plate loading performance, valid partition numbers and measured concentrations were very similar for both dPCR Nanoplates set up manually and on the epMotion.

Conclusion

The results demonstrate that automating Nanoplate setup on the epMotion 5073 preserves analytical performance while improving workflow efficiency. Sample loading, valid partition numbers, and target concentrations were highly comparable between manual and automated runs for 8.5k and 26k Nanoplate formats.

At the same time, direct dispensing into the Nanoplate reduced setup time, tip and plastic consumption, and showed no evidence of cross-contamination. Overall, epMotion 5073 provides a compact, versatile solution to standardise QIAcuity dPCR setup and reduce hands-on time in routine laboratories.

References

Paper

- [1] Scimone C, Pepe F, Russo G, Palumbo L, Ball G, Morel P, Russo A, Troncone G, Malapelle U. Technical evaluation of a novel digital PCR platform for detecting EGFR/KRAS mutations in NSCLC archived plasma specimens. The Journal of Liquid Biopsy. 2024;3:100133. doi: 10.1016/j.jlb.2023.100133. PMID: 40026570 PMCID: PMC11863824
- [2] Automation of the QIAGEN® QIAcuity Digital PCR Nanoplate Setup Using the epMotion®. White Paper No. 122 www.eppendorf.com

Ordering information Eppendorf products

Description	Order no. International	Order no. North America
epMotion® 5073I	5073000110	
TM 10-8 eight-channel dispensing tool	5280000304	
TM 50-8 eight-channel dispensing tool	5280000215	960001044
TS 50 single-channel dispensing tool	5280000010	960001010
epT.I.P.S.® Motion, 10 µL, PCR Clean, with filter	0030014391	
epT.I.P.S.® Motion, 50 µL, PCR Clean, with filter	0030014413	
epMotion® Height Adapters 40 mm	5075755009	
Thermorack for 24 tubes, 1,5 - 2mL	5075771004	
Eppendorf twin.tec® PCR Plate 96, skirted	0030128648	
Eppendorf Safe-Lock Tubes 1,5 mL	0030123328	

Ordering information QIAGEN products

Description	Cat. No	Description	Cat. No
QIAcuity One, 2plex ¹	varies	QIAcuity Nanoplate 8.5k 24-well (10)	250011
QIAcuity One, 5plex ²	varies	QIAcuity Nanoplate 8.5k 96-well (10)	250021
QIAcuity Four	varies	QIAcuity Probe PCR Kit (1 mL) ³	250101
QIAcuity Eight	varies	QIAcuity EG PCR Kit (1 mL) ⁴	250111
QIAcuity Nanoplate adapter	9027242	QIAcuity OneStep Advanced Probe Kit (1 mL) ⁵	250131
QIAcuity Nanoplate 26k 24-well (10)	250001	QIAcuity High Multiplex Probe PCR Kit (1 mL) ⁶	250133

¹ enabling up to 4plex analysis operating under software version 3.1 or higher
² enabling up to 12plex analysis operating under software version 3.1 or higher
³ 4, 5, 6 various kit sizes are available

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