Application Note

Assessment of the precision and sensitivity of QIAcuity® nanoplate digital PCR vs. qPCR

Introduction

Quantitative PCR (qPCR) measures the amplification process at each cycle of PCR. The quantification cycle (Cq) value is the number of cycles required to generate a fluorescent signal significantly different from the background fluorescence and is plotted against a standard curve with known target concentrations to provide a relative quantification. The resulting data can be highly variable due to reliance on standard curves or references to perform quantification. This is especially problematic when trying to measure low-abundance targets or small fold-changes accurately or when amplification efficiency is reduced by the presence of any PCR inhibitors introduced by the template.

Digital PCR (dPCR) works by dividing a bulk qPCR-like reaction mixture into a large number of individual reactions and then measuring the endpoint fluorescence of each partition to determine the presence or absence of the target. This makes digital PCR non-reliant on the kinetics of the PCR reaction and eliminates the dependency on standard curves, thereby enabling absolute quantification.

Here we compared the performance of qPCR and the nanoplate dPCR techniques with respect to precision and sensitivity.

Experimental setup and comparison of performance

Precision

Precision defines the closeness of repeated measurements under identical conditions to each other. We compared the precision performance of 24 qPCR and dPCR technical replicates from their respective PCR master mixes (QIAcuity Probe PCR Mix for dPCR and TaqPath™ ProAmp™ Master Mix for qPCR) spiked with varying final concentrations (200, 50 and 2.5 copies/µl) of human genomic DNA isolated with the FlexiGene® DNA Kit. Digital PCR reactions were performed on the QIAcuity One, 5plex System in QIAcuity Nanoplate 8.5K, 96-well for moderate copy number and QIAcuity Nanoplate 26K, 24-well for the low copy number using a TaqMan® PCR assay targeting the human ERBB2 gene from genomic DNA. The qPCR reaction was performed in a 96-well plate on the Applied Biosystems® 7500 Real-Time PCR System, and the measurements were quantified against a standard curve. The standard curve was done in triplicates, using 4 template amounts, ranging from 500–1 copy/µl ($R^2 = 0.999$). The standard QIAcuity probe cycling parameters were used in dPCR, while the kit manufacturer's recommendations have been followed for qPCR. The two primers and the FAM labeled probe were identical in both cases. ▷

Quantification of 24 replicates generated by each technique showed significantly less deviation from the mean value for dPCR than qPCR (Figure 1), lowering the standard deviation of the replicates down to three-fold.

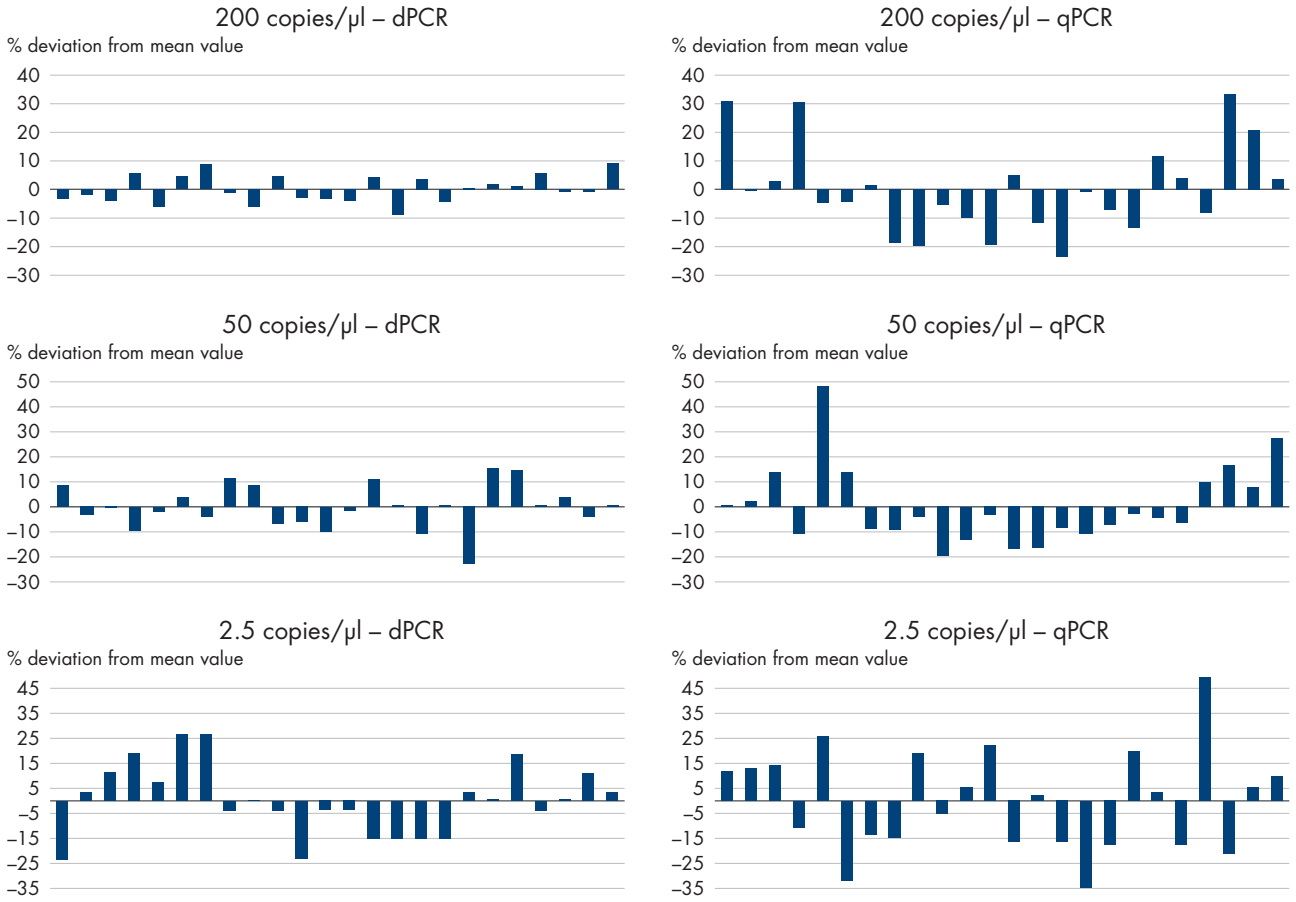

Figure 1. dPCR shows greater precision compared to qPCR for quantifying target under study. Graphs showing the quantification of 24 replicates generated by dPCR for moderate copy number (200 and 50 copies/µl) in QIAcuity Nanoplate 8.5K, 96-well and low copy number (2.5 copies/µl) in QIAcuity Nanoplate 26K, 24-well on the QIAcuity One, 5plex System (panels on the left) and by qPCR in a 96-well plate on the Applied Biosystems 7500 Real-Time PCR System (panels on the right).

Sensitivity of mutation detection

Next, we compared the mutation detection sensitivity of 3 qPCR and dPCR technical replicates from their respective PCR master mixes (QIAcuity Probe PCR Mix for dPCR and TaqPath ProAmp Master Mix for qPCR) spiked with a final concentration of 2000 copies/µl of DNA template, containing either WT or T790M mutation (MUT) of the EGFR gene in variable ratios. Digital PCR reactions were performed on the QIAcuity One, 5plex System in QIAcuity Nanoplate 26K, 24-well using a dPCR LNA Mutation Assay for EGFR p.T790M mutation. The qPCR reaction was performed in a 96-well plate on the Bio-Rad® CFX96 Real-Time PCR System, and the measurements were quantified against a standard curve for WT and MUT, respectively.

The standard QIAcuity probe cycling parameters were used in dPCR, and probes were labeled with HEX for WT and FAM for MUT in both cases.

Quantification of 3 replicates generated by each technique demonstrated higher sensitivity for dPCR compared to qPCR. The qPCR results show a loss of reliable quantification starting at a 10% mutation rate while a mutation rate down to 0.1% could be reliably detected by dPCR (Figure 2 A-B and Table).

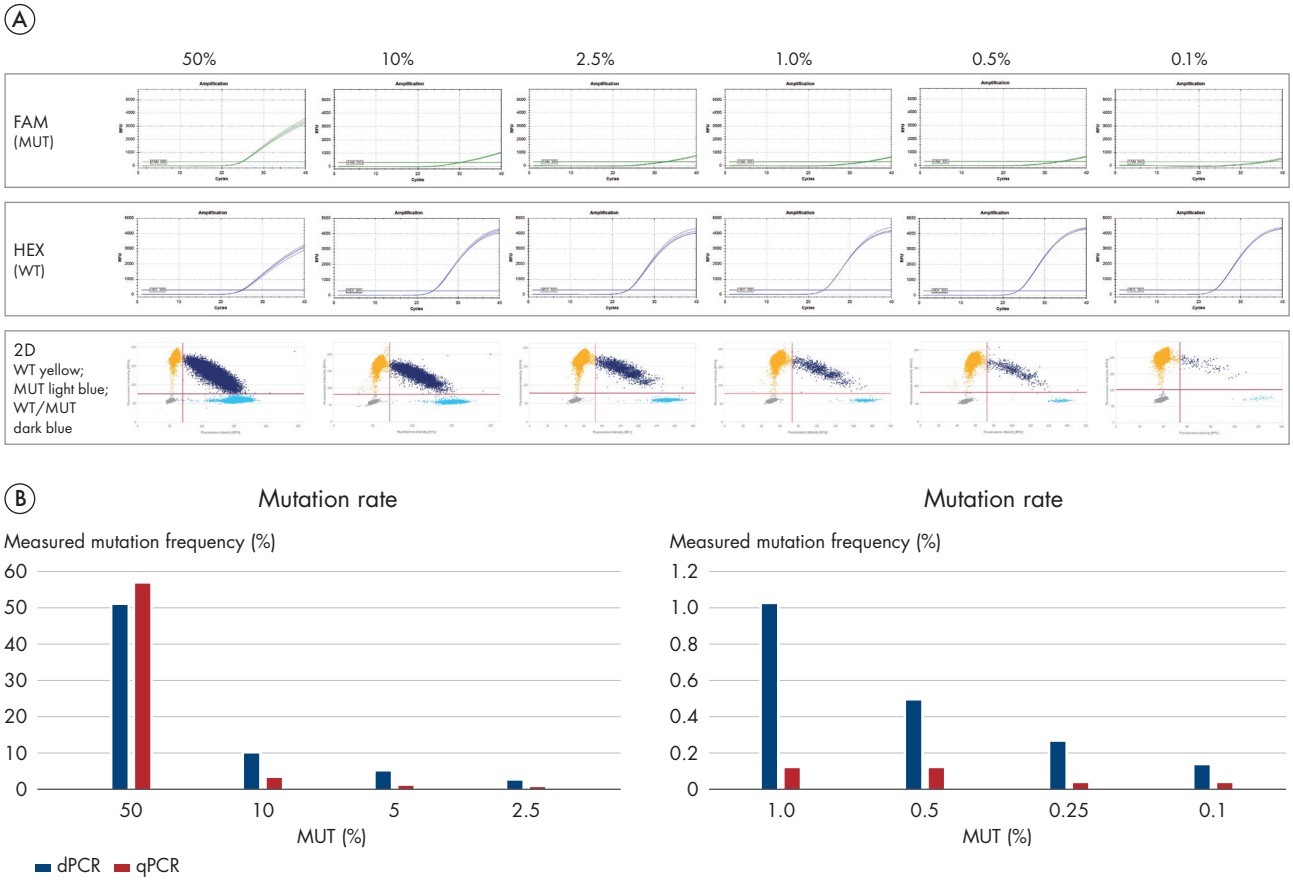

Figure 2. dPCR shows higher mutation detection sensitivity compared to qPCR. A Exemplary dPCR 2D scatterplots against the qPCR amplification curves showing a loss of reliable quantification with qPCR already at 10% mutation rate. **B** dPCR accurately detects low mutation frequencies, down to 0.1%.

Table 1. Summary of percentage mutation detected – qPCR vs. dPCR.

	Expected copy/µl (MUT)	qPCR measured copy/µl (MUT)	dPCR measured copy/µl (MUT)	qPCR measured mutation rate (%)	dPCR measured mutation rate (%)
MUT 100%	2000	1783.75	2091.3	100.00%	99.97%
MUT 50%	1000	835.05	1050.5	54.19%	50.19%
MUT 10%	200	81.82	191.8	5.09%	9.24%
MUT 5%	100	27.44	104.7	1.50%	4.98%
MUT 2.5%	50	4.86	52.6	0.27%	2.49%
MUT 1%	20	1.94	21.4	0.11%	1.02%
MUT 0.5%	10	2.25	10.1	0.12%	0.49%
MUT 0.25%	5	0.60	5.0	0.03%	0.26%
MUT 0.1%	2	0.49	2.6	0.03%	0.13%

Conclusion

The nanoplate-based digital PCR method on the QIAcuity significantly improved precision when measuring copy number states and sensitivity of mutation detection through absolute quantification and reduced standard error. This is expected to be advantageous in various applications, including copy number variation analysis, small fold-change and rare mutation detection.

The QIAcuity is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit instructions for use or user operator manual. QIAGEN instructions for use and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services (or your local distributor).

To learn more about QIAcuity nanoplate digital PCR, visit: **www.qiagen.com/applications/digital-pcr**