Experimental flexibility for a duplex *BCR-ABL* assay using the QuantStudio MAP16 Digital PCR Plate

Highlights

- Microfluidic array plate (MAP) technology provides experimental flexibility to use a single consumable up to four times
- Annealing/extension time of a duplex *BCR-ABL1* assay can be optimized with just one MAP plate
- Consistent digitization is maintained for iterative use

MAP technology utilizes fixed microchamber arrays and positive pneumatic pressure to distribute, or digitize, reagents for digital PCR (dPCR), as opposed to other methods that use fluid shearing to generate droplets. The Applied Biosystems™ QuantStudio™ MAP16 Digital PCR Plate consists of a 4 x 4 grid of dPCR arrays, each of which contains 20,480 fixed microchambers. The plate was designed to allow flexibility—up to 16 samples or as few as 4 dPCR arrays (one column) can be loaded and run simultaneously without sacrificing data quality. This flexibility can be useful for applications in which lower dPCR throughput or iterative assay optimization, as we describe here, is required.

Method

In this experiment, an iterative test was performed with a single QuantStudio MAP16 plate to optimize the extension time of a *BCR-ABL1* assay for detecting a gene fusion that occurs in 95% of chronic myeloid leukemia patients. To showcase the experimental flexibility of the consumable for repeated use, we ran four sequential dPCR runs with extension steps lasting 0, 15, 30, and 45 seconds. We compared the final calculated target concentrations and the fluorescence intensity across conditions. To evaluate the integrity of the QuantStudio MAP16 plate across successive runs, we calculated the total number of microchambers analyzed for each experimental condition.

We selected the BCR-ABL pDNA calibrant (Sigma, Cat. No. ERMAD623), a plasmid that contains target sequences for both *BCR-ABL1* and *ABL1*. European reference and measurement (ERM) certification of this well-characterized calibrant ensures reliability and comparable results. We used a published duplex assay targeting the *BCR-ABL1* (FAM™ dye–labeled probe) and *ABL1* (HEX™ dye–labeled probe) sequences [1]. Each reaction contained a final concentration of 500 copies/µL of the target. Four replicates were run using one column at a time, and the concentration of each target was quantified in copies/µL. The reaction preparation and dPCR protocol are shown in Tables 1 and 2, respectively.

Table 1. dPCR reaction preparation.

Reagent	Final concentration
Absolute Q DNA Digital PCR Master Mix	1X
BCR-ABL1 assay (20X, FAM dye–conjugated)	1X
ABL1 assay (20X, HEX dye–conjugated)	1X
BCR-ABL pDNA	500 copies/µL

Table 2. Thermal protocol for dPCR on the Applied Biosystems™ QuantStudio™ Absolute Q™ dPCR system.

Step	Temperature	Time	Cycles
Hot start	96°C	5 min	1
Denature	96°C	5 sec	40
Anneal/ extend	61°C	Variable (0–45 sec)	

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In each microchamber, both low reagent volume and close proximity to the heated surface contribute to PCR robustness over a range of extension times. The suggested extension time is typically one minute per 1,000 bases. In this study, we tested the performance of the *BCR-ABL1* duplex assay as we increased the extension time. In each of four successive runs, a different column was utilized to evaluate the effects of changing the duration of the annealing/extension step. We started at 0 seconds and increased the time by 15 seconds in each subsequent run (Figure 1).

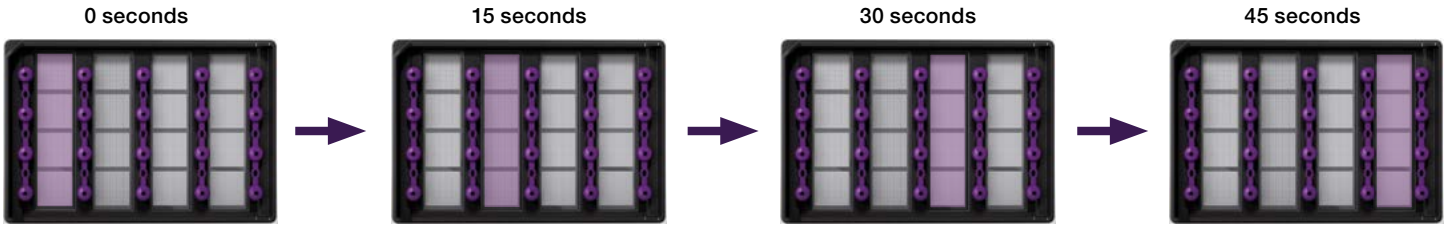

Figure 1. The QuantStudio MAP16 plate was used four times with one column per experiment to optimize the annealing/extension time for a FAM/HEX multiplex assay.

Results

The quantitative results for the *BCR-ABL1* (FAM) and *ABL1* (HEX) targets across the four extension times are shown in Figure 2 along with representative 2D scatter plots. Extension times of 15 seconds or longer resulted in accurate quantification, while extension times of 30 seconds or longer provided the best separation between positive and negative microchamber clusters.

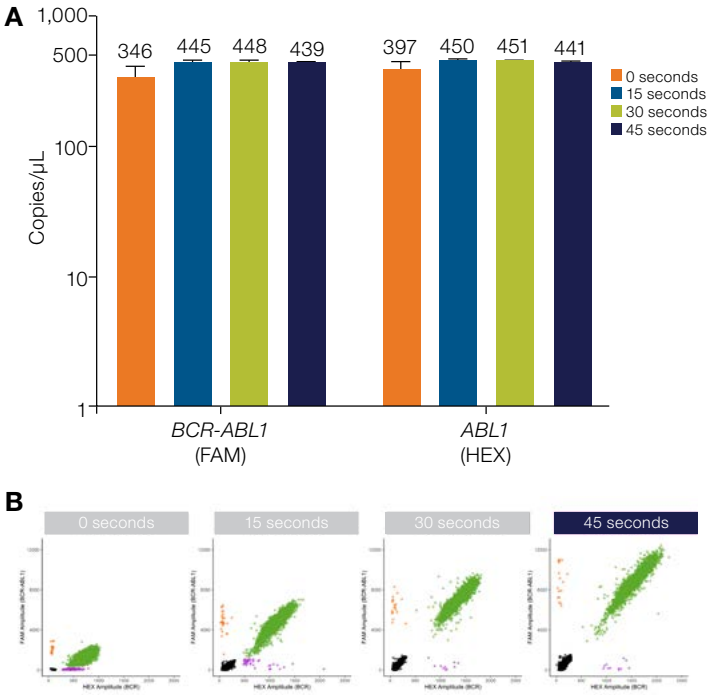

Figure 2. Comparison of quantitative results across extension times. (A) Concentrations of multiplex assay targets in the FAM and HEX channels. The error bars represent standard deviation, and mean values are noted above the bars. **(B)** Two-dimensional dPCR scatter plots for representative reactions under the study conditions. The extension time is indicated above each plot.

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The industry standard for the minimum number of analyzed dPCR microchambers is typically 20,000. In addition to consistent quantification with repeated use of the same QuantStudio MAP16 plate, the average total number of microchambers analyzed per unit remained well above the targeted minimum at 20,252 (standard deviation: 165) per reaction. Figure 3 shows the average number of accepted microchambers and associated standard deviation for the entire plate, as well as the average per run. Since each dPCR run for this assay required 40 PCR cycles, the microchambers in the last column had been exposed to thermal changes for a total of 160 cycles after the fourth run. The QuantStudio MAP16 plate consistently yielded acceptable numbers of microchambers that were well above 20,000 per dPCR array, even in later runs (Figure 3).

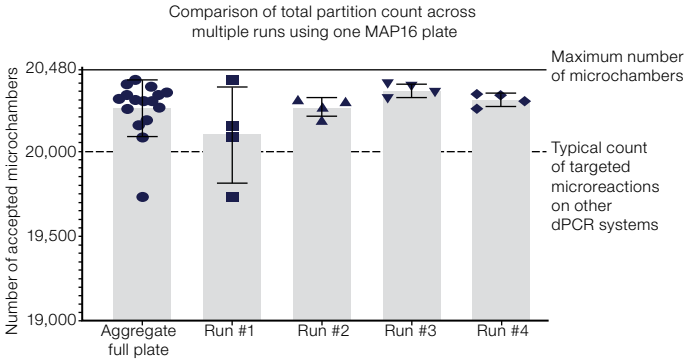

Figure 3. Comparison of total microchamber counts for columns run sequentially on a single QuantStudio MAP16 plate. The QuantStudio MAP16 plate was run four separate times to test the effect of extension time on dPCR assay performance. The average count for all four runs is shown in the first column, and the results of individual runs are shown in subsequent columns. Each point represents the total microchamber yield from one dPCR array.

Summary

MAP technology enhances dPCR. With a simple workflow and highly consistent performance, MAP technology allows flexibility in experimental design and optimization of dPCR assay conditions without sacrificing robustness.

Reference

1. Alikian M, Whale AS, Akiki S et al. (2017) RT-qPCR and RT-digital PCR: a comparison of different platforms for the evaluation of residual disease in chronic myeloid leukemia. *Clin Chem* 63(2):525–531.

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