Sensitive & flexible Crystal Digital PCR™ quantification using the naica® multiplex PCR MIX

A defining characteristic of digital PCR leading to its high sensitivity is the partitioning of the PCR reaction mix into thousands of individual compartments separated by an isolating phase. Chemical interactions occurring at the interface between the reaction mix and the isolating phase can dramatically impact PCR efficiency. The composition and performance of the PCR master mix are critical to ensure efficient amplification of the targets and partition compatibility. The naica® multiplex PCR MIX was specially formulated to provide robust partition compatibility and excellent linear Crystal Digital PCR™ quantification of multiple targets across the entire dynamic range of the naica® system, even in the presence of a complex sample DNA background. To ensure the flexibility and sensitivity that our customers need, the naica® multiplex PCR MIX is available at both 5X and 10X concentrations, thus freeing up valuable reaction volume that can be instead occupied by additional sample input, probes and/or primers.

The naica® multiplex PCR MIX ensures simultaneous linear quantification of multiple targets across the dynamic range of the naica® system

The naica® multiplex PCR MIX was optimized for use on the naica® system to ensure compatibility with dual-labelled fluorescent probe multiplex assays. A stably incorporated blue channel reference dye ensures reliable partition detection and facilitates reaction assembly. Using the 10X naica® multiplex PCR MIX, simultaneous detection of three reference targets from 0.2 to 13,000 copies (cp)/µL was achieved on the naica® system (**Figure 1**). The high initial concentrations of the mix (available at both 5x and 10x concentrations) maximize the reaction volume available for sample input, thus increasing the potential to detect targets in dilute samples.

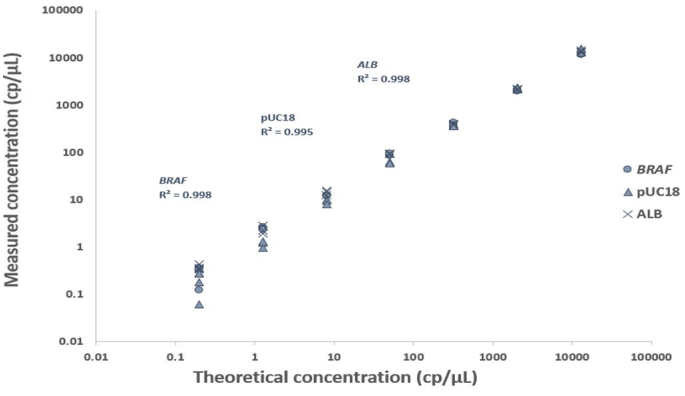

Figure 1 | Linear and sensitive 3-color Crystal Digital PCR™ quantification across the full dynamic range of Sapphire chips using naica® multiplex PCR MIX. Serial dilutions from 0.2 to 13000 cp/µL of human genomic (hg)DNA and pUC18 plasmid were assessed in triplicates in a 25 µL reaction. Human BRAF and Albumin (ALB) genes were detected in the blue and red channels, respectively, while plasmid pUC18 was detected in the green channel. Coefficient of determination scores of R^2>0.99 show highly reliable results for each target. The concentrations of each dilution point were: 0.2, 1.5, 8.0, 50, 320, 2050, and 13000 cp/µL.

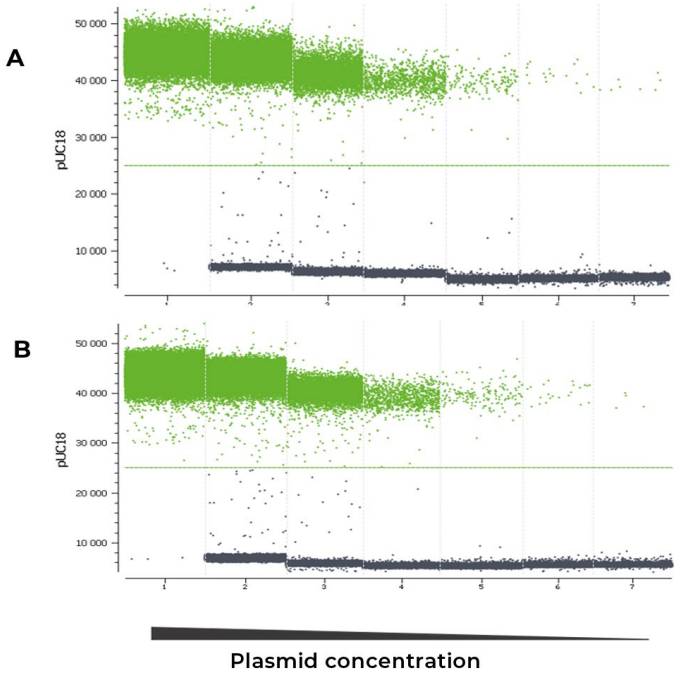

Figure 2 | Fluorescence 1D-dotplots generated by Crystal Miner software show the dynamic range from 13000 to 0.2 cp/µL of pUC18 DNA amplification in the presence of (A) 3000 cp/µL (10ng) of hgDNA template (triplex reaction) and (B) no additional DNA templates (simplex reaction). The pUC18 concentrations of each dilution point were: 13000, 2050, 320, 50, 8.0, 1.5, and 0.2 cp/µL. Note: both the simplex and the triplex reactions contain the primers and probes corresponding to the pUC18, BRAF and ALB target sequences.

Stable and sensitive detection of a low concentration target in a multiplex assay

Co-amplification of several targets in a multiplex assay is more complex than a single amplification reaction due to potential crosstalk and reaction complexity, possibly reducing the linear range of detection. Using the naica® multiplex PCR MIX, the pUC18 target was equally and comparably quantified throughout the dynamic range of the naica® system using Sapphire chips in both a triplex assay (**Figure 2A** and **3A**) in which both the BRAF and ALB hgDNA targets were present in high concentrations, and a simplex assay (**Figure 2B** and **3B**) in which pUC18 template was the unique DNA present in the reaction. In addition, the fluorescence levels and separability of the pUC18 positive and negative populations were similar in the simplex and triplex reactions (**Figure 2**). Moreover, regardless of the simplex or triplex context, the pUC18 target was robustly and reliably quantified (**Figure 3**), and the detection range and the linearity of this detection were similar in the two contexts.

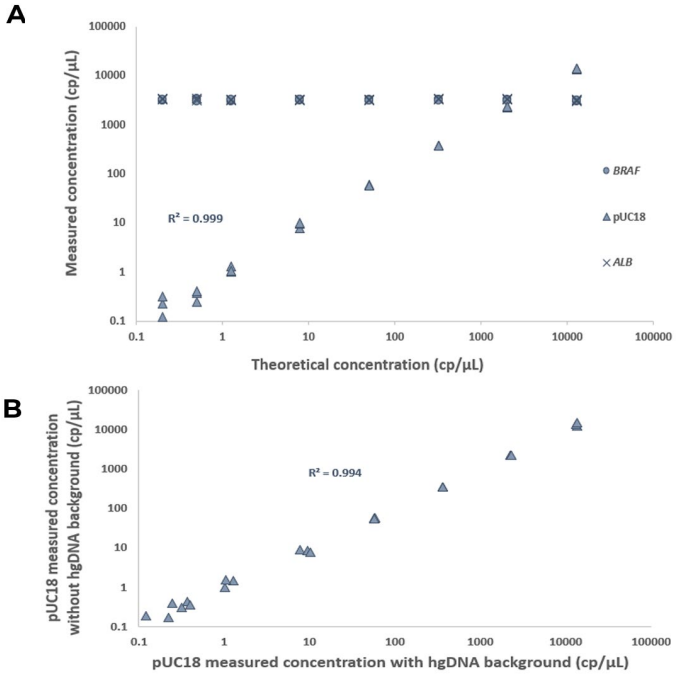

Figure 3 | Simplex versus triplex quantification with naica® multiplex PCR MIX. Serial dilutions of pUC18 plasmid from 0.2 to 13,000 cp/µL in a 25 µL reaction were quantified in triplicate in a background of (A) 3,000 cp/µL of hgDNA or (B) without additional DNA template. The quantification of the hgDNA reference (as measured through the BRAF and ALB genes) demonstrated excellent repeatability, with relative standard deviations of 2.3% and 2.5%, respectively (n= 21).

Technical Note Highlights

The naica® multiplex PCR MIX enables an excellent linear Crystal Digital PCR™ quantification of multiple targets across the entire dynamic range of the naica® system.

Using the naica® multiplex PCR MIX, a target is equally and comparably quantified in both a simplex and a triplex detection assay.

naica® multiplex PCR MIX is available at both 5X and 10X concentrations, thus freeing up valuable reaction volume that can be instead occupied by additional sample input, probes and/or primers.

To learn more about digital PCR, please visit Stilla Technologies' Learning Center at **stillatechnologies.com/digital-pcr**

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