Robust and sensitive 6-color Crystal Digital PCR™ using the naica® multiplex PCR MIX

Multiplexed digital PCR allows the simultaneous absolute quantification of several targets from a single sample. By using a combination of dual-labelled fluorescent probes, multiplexed digital PCR provides a simple solution that makes it possible to use less of a precious sample when faced with limited quantity without sacrificing sensitivity. To meet the increased need to analyze simultaneously a high number of targets with improved sensitivity, Stilla® has recently developed the naica® multiplex PCR MIX, an easy-to-use PCR mastermix specially formulated to ensure optimal Crystal Digital PCR™ multiplexing on the naica® system. This technical note focuses on the excellent reliability of the 10X naica® multiplex PCR MIX for sensitive highplex DNA quantification using the new 6-color naica® system for Crystal Digital PCR™.

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

Optimized for multiplexed Crystal Digital PCR™ detection of DNA with dual-labelled fluorescent probes, the naica® multiplex PCR MIX provides accurate absolute quantification on the naica® system. W hen combining the 10X naica® multiplex PCR MIX with the 6-color naica® system, we obtained R^2 scores > 0.99 for all six targets ranging from 0.2 to 13000 copies (cp)/µL, highlighting the reliable simultaneous quantification of six different targets in six independent fluorescence channels (**Figure 1**).

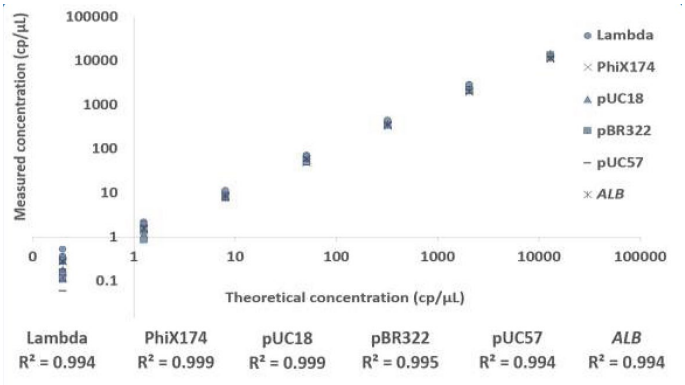

Figure 1 | Linear and sensitive 6-color Crystal Digital PCR™ results across the full dynamic range of Sapphire chips using the 10X naica® multiplex PCR MIX. Variable concentrations ranging from 0.2 to 13000 cp/µL of a DNA containing six different template sources were assessed in triplicate in 25 µL Sapphire chip reactions. The amplification of each of the six indicated DNA was individually detected in blue, teal, green, yellow, red and infra-red channels. Coefficients of determination R^2 > 0.99 showed highly reliable results for all assessed targets. The concentrations of each dilution point were: 0.2, 1.5, 8.0, 50, 320, 2050 and 13000 cp/µL.

In a highplex reaction setup, the use of a high number of probe and primer sets limits the sample volume that can be included in the reaction. To maximize the sample input volume, we have optimized the naica® multiplex PCR MIX to be stable as a 10X concentrate, resulting in the use of 50 to 80% less volume compared to 2X and 5X digital PCR mixes. This gain in sample volume input is especially valuable when detecting rare targets

Robust quantification of low target concentrations in a highly abundant and complex DNA background

A major advantage of digital PCR is its capacity to detect a low concentration target in the presence of multiple additional target amplifications. To assess the highplex robustness of the naica® multiplex PCR MIX across the dynamic range of the 6-color naica® system using Sapphire chips, two 6-color Crystal Digital PCR™ assays have been tested. Each assay contains variable concentrations from 0.2 to 13000 cp/µL of one DNA target in in a background of five additional targets each at 3000 cp/µL. In the most concentrated DNA setup assayed among the different test conditions, a target at an initial concentration of 13000 cp/µL is expected to be present at an average of 9 copies per partition in a single Sapphire well just after partitioning. More than 99.9% of the partitions are expected to contain at least one copy of the target. W hen multiplexed with five additional targets each at a concentration of 3000 cp/µL, each partition is expected to contain on average 19 amplifiable DNA copies. For all tested concentrations spanning four orders of dynamic range, excellent linear quantifification was observed (Fig. 2A and 2C). These results were comparable to those obtained when the same DNA targets were individually amplified across the same set of variable concentrations and in the absence of all other DNA target amplifications (Fig. 2B and 2D). These results demonstrate the robust and highly sensitive highplex performance of the naica® multiplex PCR MIX when combined with 6-color Crystal Digital PCR™.

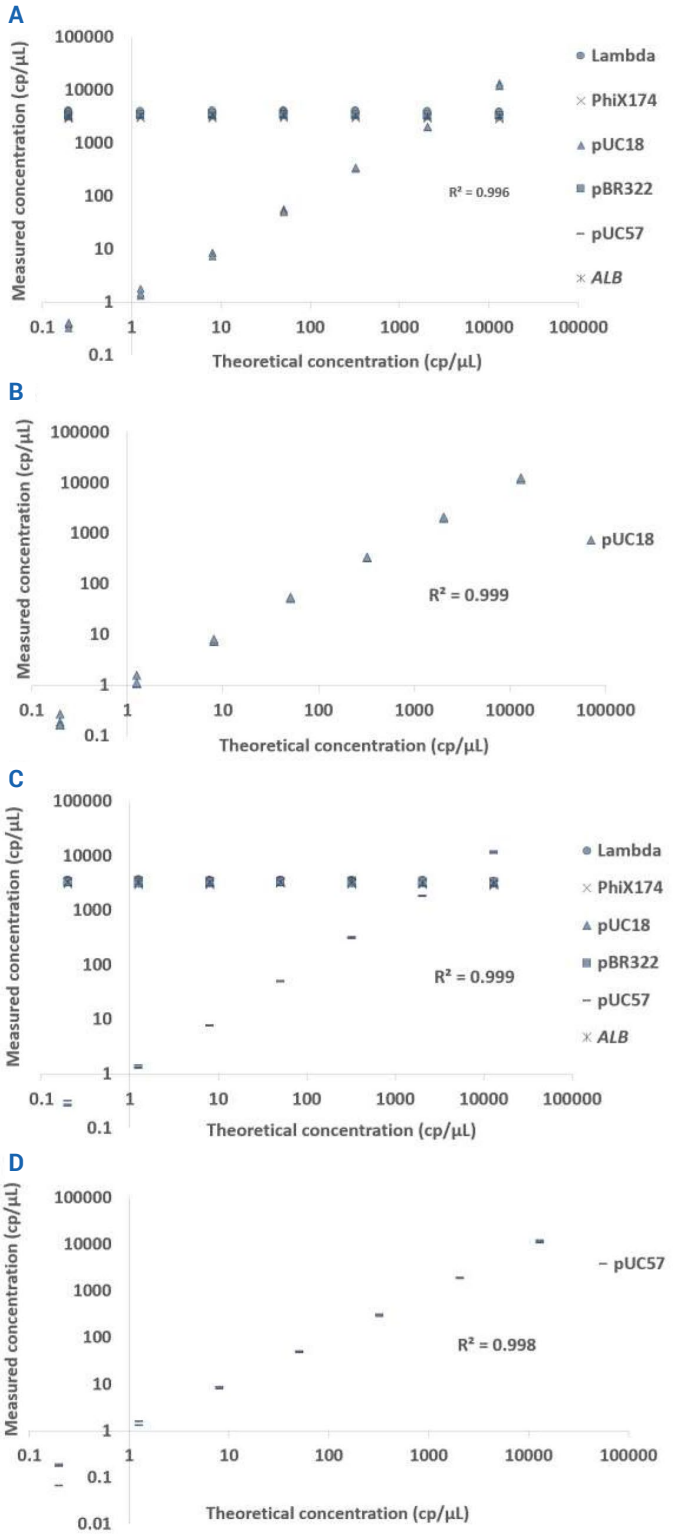

Figure 2 | Robust highplex amplification using the naica® multiplex PCR MIX. Serial dilutions from 13000 to 0.2 cp/µL of pUC18 plasmid (A and B) or a custom pUC57 plasmid (C and D) were quantified in triplicate in a background of five additional amplified targets each at 3000 cp/µL (A, C) or in the absence of other DNA targets (B, D). Coefficients of determination $R^2 > 0.99$ showed reliable results for all targets independent of the multiplex context. The relative standard deviations for each of the five additional targets remained between 2.3% and 3.1% (n=21), demonstrating excellent repeatability.

Technical Notes Highlights

- The naica® multiplex PCR MIX: ensures robust and repeatable highplex Crystal Digital PCR™ quantification;
- enables an excellent linear quantification of six independent DNA targets across the complete dynamic range of the 6-color naica® system on Sapphire chips;
- is available as a 10X concentrate, ensuring highplexing flexibility on the naica® system;
- 10X concentrate uses 50% less volume than a 5X PCR mix, allowing for more DNA volume to be loaded. This gain in volume is especially valuable when detecting rare targets.

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

MKT-00067 Rev. A