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The Next-Generation PCR-Based Quantification Method for Ambient Waters: Digital PCR

Yiping Cao, John F. Griffith, and Stephen B. Weisberg

Southern California Coastal Water Research Project, Costa Mesa, CA

ABSTRACT

Real-time quantitative PCR (qPCR) is increasingly being used for ambient water monitoring, but development of digital polymerase chain reaction (digital PCR) has the potential to further advance the use of molecular techniques in such applications. Digital PCR refines qPCR by partitioning the sample into thousands to millions of miniature reactions that are examined individually for binary endpoint results, with DNA density calculated from the fraction of positives using Poisson statistics. This direct quantification removes the need for standard curves, eliminating the labor and materials associated with creating and running standards with each batch, and removing biases associated with standard variability and mismatching amplification efficiency between standards and samples. Confining reactions and binary endpoint measurements to small partitions also leads to other performance advantages, including reduced susceptibility to inhibition, increased repeatability and reproducibility, and increased capacity to measure multiple targets in one analysis. As such, digital PCR is well suited for ambient water monitoring applications and is particularly advantageous as molecular methods move toward autonomous field application.

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