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

Yiping Cao, John F. Griffith, Stephen B. Weisberg
Southern California Coastal Water Research Project Authority, Costa Mesa, California, USA

Abstract

Real-time quantitative PCR (qPCR) is increasingly being used for ambient water monitoring, but development of digital polymerase chain reaction (dPCR) has the potential to further advance 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 end-point 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 end-point 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, dPCR is well suited for ambient water monitoring applications, and is particularly advantageous as molecular methods move towards autonomous field application.

dPCR vs. qPCR: Basics

- qPCR quantifies a sample indirectly based on interpolation from standard curves and the assumption that sample and reference DNA amplify at the same speed/efficiency.
- dPCR quantifies a sample directly by counting frequency of positives among the thousands/millions of miniature partitions of the sample itself.

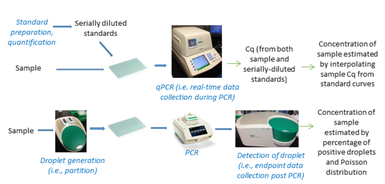

Figure 1. Workflow comparison of qPCR and digital PCR. Note that a variety of platforms exist for both qPCR and digital PCR. For ease of presentation, a 96-well qPCR platform (CFX96, Bio-Rad Laboratories) and a 96-well droplet digital PCR system (QX100, Bio-Rad Laboratories) are used as examples here.

dPCR outperforms qPCR among many performance metrics

- **Accuracy**
  - qPCR can be highly biased due to variability in standard reference material and mismatched amplification efficiency between reference and samples.
  - dPCR quantification is not affected by variability in standards and amplification efficiency.
  - After correcting for bias in qPCR standards, and in the absence of inhibition, qPCR and digital PCR typically provide comparable results (Figure 2) [1].
- **Precision**
  - dPCR has superior repeatability and reproducibility because its quantification is not affected by delayed amplification or variability in Cq values or variability associated with qPCR standards.
- **Sensitivity**
  - dPCR has higher nominal sensitivity because it tolerates higher levels of inhibitors and is less affected by background non-target DNA than qPCR.

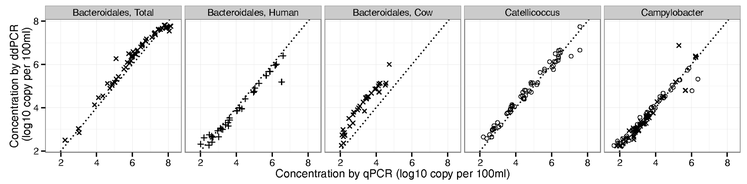

Figure 2. Comparison of ddPCR and qPCR results for quantifying general fecal indicator bacteria (total Bacteroidales), host-associated fecal markers (Bacteroidales associated with human and cow fecal material, Catellicoccus associated with gull fecal material), and pathogens (Campylobacter). Symbols indicate ambient freshwater samples spiked with cow (x-cross), gull (circle) feces, and sewage (cross). The dotted line denotes the 1:1 line. See Ref [1] for primer and probe sequences and references.

- **Tolerance to PCR inhibition**
  - Increased tolerance to inhibition by dPCR, attributed mostly to the binary nature of dPCR quantification, has been reported across many studies [1].
  - Such tolerance however many vary depending on type/ concentration of inhibitors, and reagents used in different dPCR systems.
- **Capacity to multiplex**
  - qPCR multiplexing requires complex optimization and can be severely biased compared to simplex reactions.
  - dPCR multiplexing produces quantification consistent with simplex reactions (Figure 3) [2].
- **Time to results:** No appreciable difference.
- **Cost**: Conclusions on cost comparison are variable across studies, and platform dependent.

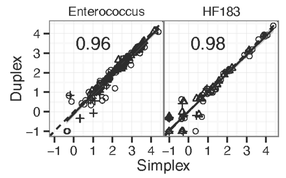

Figure 3. Comparison of duplex and simplex ddPCR quantification of *Enterococcus* and the HF183 human-fecal marker in fecal (circles) and water (freshwater: triangles; marine water: crosses) samples. Regression lines (solid lines), their standard errors (grey shading), and corresponding correlation coefficients are as displayed.

dPCR limitations

- dPCR has a smaller dynamic range of quantification than qPCR.
- dPCR quantification may be biased if uniform partition of quantification targets is not achieved.

dPCR is uniquely suited for ambient water monitoring

Unique challenges/needs in ambient water monitoring	dPCR suitability
Need to detect rare targets in complex environmental matrices	Higher tolerance to inhibition, higher nominal sensitivity, higher precision especially at low target concentrations compared to qPCR
Great need to discern and/or compare temporal and spatial patterns, amidst potential analytical method variability	High repeatability and reproducibility afforded by digital PCR within runs, between runs, operators, and laboratories
Strong desire for autonomous real-time monitoring systems that eliminate delays caused by sample transport	Elimination of need to run standard curves in the field Reduction in complexity and cost for autonomous sytems due to end-point detection instead of continuous signal detection as in qPCR

References

1. Cao, Y., Griffith, J.F. and Weisberg, S.B. 2016. The next generation PCR-based quantification method for ambient waters: Digital PCR. *Methods in Molecular Biology Series: Marine Genomics*. Bourlat, S.J. (ed), Springer, New York, NY.
2. Cao, Y., Raith, M.R. and Griffith, J.F. 2015. Droplet digital PCR for simultaneous quantification of general and human-associated fecal indicators for water quality assessment. *Water Research* 70, 337-349.