Digital PCR

Discover powerfully simple digital PCR

Learn about the benefits of dPCR and how it differs from real-time PCR

What is digital PCR?

Digital PCR (dPCR) is a specialized approach to nucleic acid detection and quantification that estimates absolute numbers of molecules through statistical methods. dPCR uses the same fundamental chemistry as real-time PCR (qPCR), but unlike qPCR data, dPCR data is collected at the endpoint of the reaction mix.

Before amplification, a bulk PCR reaction made up of nucleic acid, primers, probes, and master mix is digitized into many thousands of nanoliter-sized microreactions. As this digitization process distributes the PCR mix across so many microreactions, each microreaction will effectively either contain zero or one of the target nucleic acid molecules. The isolated microreactions are then amplified, and data is collected from each microreaction at the end of the thermal cycling process. Microreactions that do not contain the target will not show postamplification fluorescence, while those that do contain the target will show postamplification fluorescence.

In effect, the original reaction is turned into many binary reactions. After counting the positive microreactions, simple statistics can be used to then determine the “absolute” quantity of the target molecule rather than a quantity estimated by comparison to a standard of known concentration. This technology offers an alternative to qPCR for absolute quantification and rare-allele detection, and it does not rely on the number of amplification cycles to determine the initial amount of template nucleic acid in each sample.

Figure 1 shows the reproducibility of dPCR for absolute quantification of a target. It shows the results of an absolute quantification experiment run on 24 plates across three different users. As you can see, these three different users were able to obtain highly reproducible data.

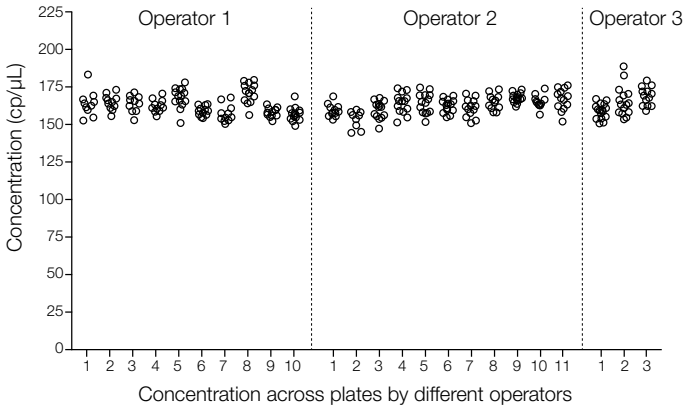
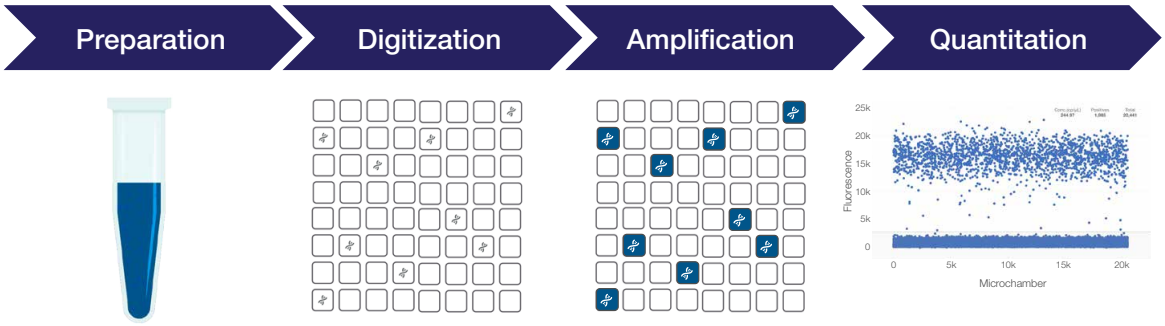


Figure 1. Reproducibility of dPCR data across three operators using 24 different plates.



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Digital PCR vs. real-time PCR vs. traditional PCR

To date, qPCR has been a powerful and sensitive gene analysis technique used for a broad range of applications. As the name suggests, qPCR measures PCR amplification as it occurs, unlike traditional PCR, which collects results after the reaction is complete, making it impossible to determine the starting concentration of nucleic acid. qPCR's entry into the market revolutionized PCR-based quantitation of DNA and RNA.

dPCR is a newer approach to nucleic acid detection and quantification that estimates absolute numbers of molecules through statistical methods. dPCR is well suited to performing rare allele detection, measurement of copy number variation, viral titer measurement, quantification of next-generation sequencing libraries, and detecting rare targets from environmental samples such as wastewater.

Digital PCR vs. real-time PCR vs. traditional PCR at a glance

	Digital PCR	Real-time PCR	Traditional PCR
Overview	Measures the fraction of negative microreactions to determine the absolute number of copies	Measures PCR amplification as it occurs in a bulk reaction mix	Measures the amount of accumulated PCR product at the end of the PCR cycle
Quantitative?	Yes—the fraction of negative microreactions is fit to a Poisson statistical algorithm	Yes—the quantity of the PCR product is directly proportional to the amount of template nucleic acid (standard curve)	No—however, comparing the intensity of the amplified band on a gel to standards of a known concentration can give semiquantitative results
Applications	• Absolute quantification of viral load • Absolute quantification of nucleic acid standards • Absolute quantification of next-generation sequencing libraries • Rare-allele detection • Absolute quantification of gene expression • Analysis of multiple targets on a single molecule through multiplexing	• Quantitation of gene expression • Microarray verification • Quality control and assay validation • Pathogen detection • Single-nucleotide polymorphism (SNP) genotyping • Copy number variation • MicroRNA analysis • Viral quantitation • siRNA/RNAi experiments	Amplification of DNA for: • Sequencing • Genotyping • Cloning
Advantages	• No need to rely on references or standards • Desired precision can be achieved by increasing the total number of PCR replicates • More tolerant to some PCR inhibitors • Capable of analyzing complex mixtures • Provides a linear response to the number of copies present to allow small-fold changes to be detected	• Increased dynamic range of detection • No post-PCR processing • Detection is capable down to a two-fold change • Collects data in the exponential growth phase of PCR • An increase in reporter fluorescent signal is directly proportional to the number of amplicons generated • The cleaved probe provides a permanent record amplification of an amplicon	• Simple to design • Easy to perform • Uses more readily available equipment and reagents • No special training required

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