QuantStudio® 3D Digital PCR System

Absolutely attainable digital PCR

- Simple chip-based workflow—no emulsion PCR
- Affordable—low total cost of ownership
- Absolute quantification—accurate and precise detection without a standard curve

The QuantStudio® 3D Digital PCR System.

Introduction

Historically, the adoption of digital PCR was limited due to cumbersome workflows and the high cost to implement. The simple and affordable QuantStudio® 3D Digital PCR System removes these barriers, bringing digital PCR to any lab. Digital PCR expands the application boundaries of traditional real-time PCR by enabling absolute quantification without the use of a standard curve (Figure 1). With digital PCR, researchers can go beyond measuring C_t to detecting individual DNA molecules—gaining additional sensitivity and precision for a variety of experiments, including but not limited to:

- Copy number variation (CNV) analysis
- Pathogen detection and load determination
- Absolute quantification of standards
- Library quantification for next-generation sequencing
- Characterization of low-fold changes in mRNA and miRNA expression
- GMO detection and contamination assessment

life technologies

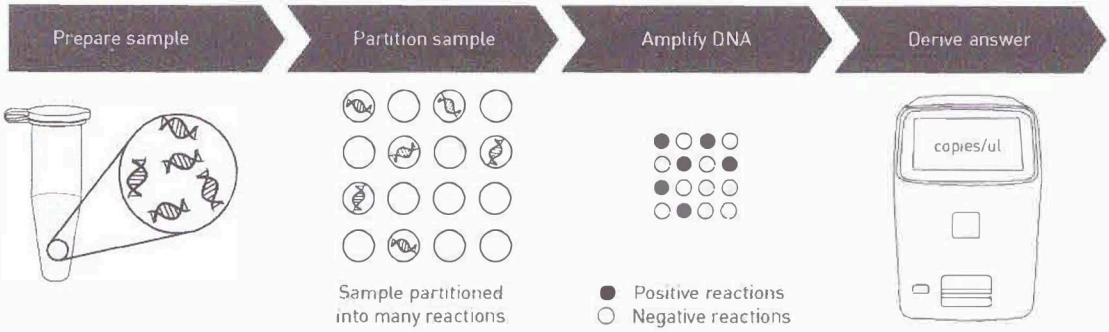

Figure 1. Digital PCR enables absolute quantification of target alleles. To perform digital PCR, a nucleic acid mixture is partitioned into many reaction wells, such that some wells receive a target molecule and some do not. Reactions are subjected to standard PCR to identify wells that have not received target molecules. A standard statistical correction model accounts for wells that may have received more than a single target molecule, and a final concentration value is produced. Given that digital PCR does not rely on C_t values to quantify copy number, comparison to a known standard is not required for absolute quantification.

Simple workflow

The QuantStudio™ 3D Digital PCR System minimizes the level of expertise needed to perform digital PCR. Simply load the reaction mix onto the uniquely tagged chip, amplify on a dual flat block thermal cycler, and read the target concentration in less than a minute on the QuantStudio™ 3D Digital PCR Instrument. Samples and amplification products remain completely contained throughout the streamlined process, and an easily interpretable answer (in target copies/µL) is produced. In comparison, current droplet-based methods involve multiple pipette transfer steps and expose the reaction mixture to the environment, which may increase the likelihood of cross-contamination and the release of amplicons to surfaces and equipment in the lab (Figure 2).

The QuantStudio™ 3D Digital PCR System leverages a high-density nanofluidic chip technology to partition a sample into as many as 20,000 independent reaction wells. Comprised of consistently sized wells etched in the solid substrate (Figure 3), the chip enables straightforward and robust sample partitioning such that thousands of data points can be analyzed in a run. In comparison, droplet methods rely upon emulsion PCR in which results can be highly variable.

The QuantStudio™ 3D Digital PCR System is capable of detecting both TaqMan® and SYBR® chemistries. However, gold-standard TaqMan® Assays are ideal for digital PCR, as TaqMan® chemistry minimizes false positives in quantitative PCR experiments and eliminates the need for melt curve analysis. We offer over 8 million predesigned TaqMan® Assays, providing a starting point for assay selection when performing digital analysis using the QuantStudio™ 3D Digital PCR System.

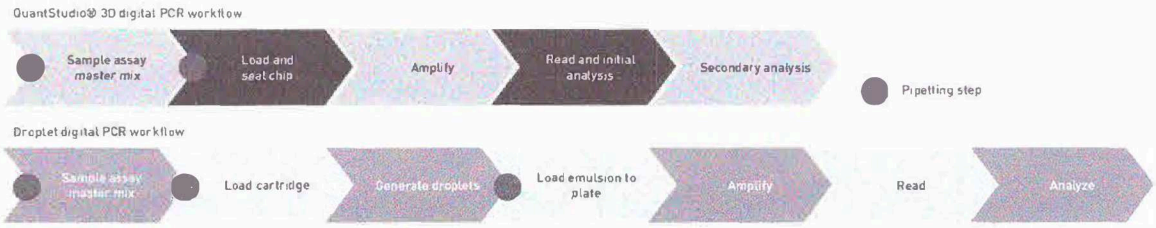

Figure 2. Chip-based digital PCR workflow: more streamlined and secure than droplet-based workflow. The QuantStudio™ 3D chip-based digital PCR workflow involves minimal pipetting steps and a sealed system (once the chip is closed). In comparison, a droplet-based approach requires more pipetting steps as well as multiple transfer steps, which expose the sample to potential contamination.

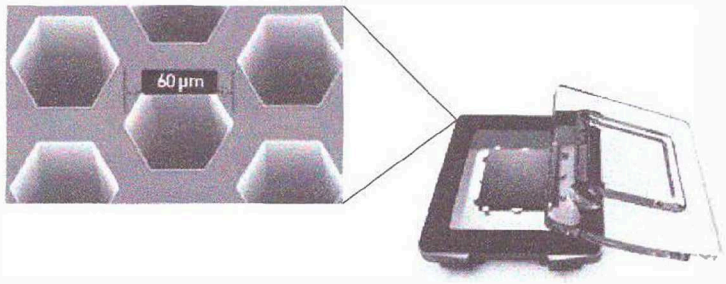

Figure 3. The QuantStudio™ 3D Digital PCR 20K Chip. Each chip contains 20,000 consistently sized reaction wells for optimal loading and is sufficient for one duplexed sample, allowing users to design experiments as needed—no more waiting for "enough" samples to fill the run.

Absolute quantification with confidence

For each answer calculated by the QuantStudio™ 3D Digital PCR System that determines absolute number (copies/µL), a data quality assessment is made. Data considered to be of marginal or failing quality are then appropriately flagged for further review in secondary analysis. AnalysisSuite™ Cloud Software supports secondary analysis from any computer connected to the Internet through a personalized, cloud-based Life Technologies™ user account. Data transfer is seamless and completely automated, if desired. Data can also be transfered by more conventional approaches such as across an internal network or via a USB flash drive.

QuantStudio™ 3D AnalysisSuite™ Software is available for further data QC and multichip analysis for specific applications. Chip data are uploaded to a secure environment and analyzed using different application modules such as absolute quantification and relative quantification, with visualizations allowing for chip inspection, clustering, and calculation of copy number values (Figure 4).

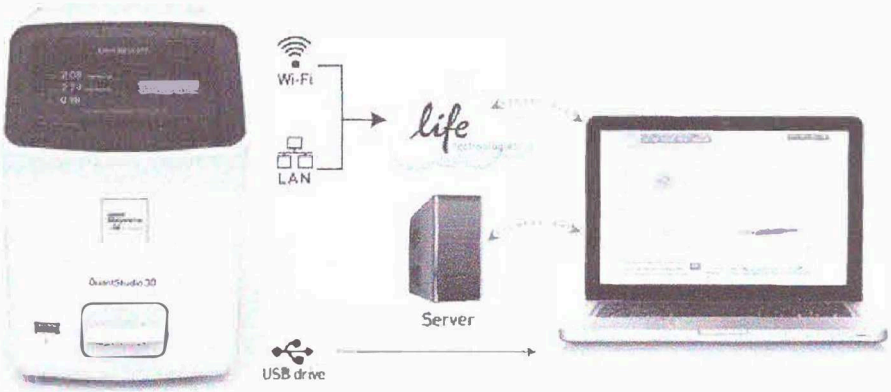

Figure 4. Primary analysis performed directly on the QuantStudio™ 3D Digital PCR Instrument in less than 1 minute. The instrument displays absolute quantification in copies/µL and a quality flag. Data files can be automatically transferred to QuantStudio™ 3D AnalysisSuite™ Software through a personalized, cloud-based Life Technologies™ user account on the Web. A stand-alone server can be used in lieu of accessing the cloud. An example of data analysis using the Relative Quantification Module in QuantStudio™ 3D AnalysisSuite™ Software is shown on the laptop screen. Ideal for applications such as rare allele detection, data from multiple chips can be combined to automatically display a scatter plot based on the color of rare and wild type events. For further quality control, boundaries of rare vs. wild type vs. undetermined events can be manually defined.

Performance to meet your application needs

High precision is a key performance attribute of digital PCR, and is driven by the number of replicates (chip reaction wells) that are run. With 20,000 independent reaction wells per chip, the QuantStudio™ 3D Digital PCR System enables high precision and high accuracy (Figures 5 and 6). This level of performance is critical for relative quantification applications such as CNV analysis, rare allele detection, and low-fold gene expression.

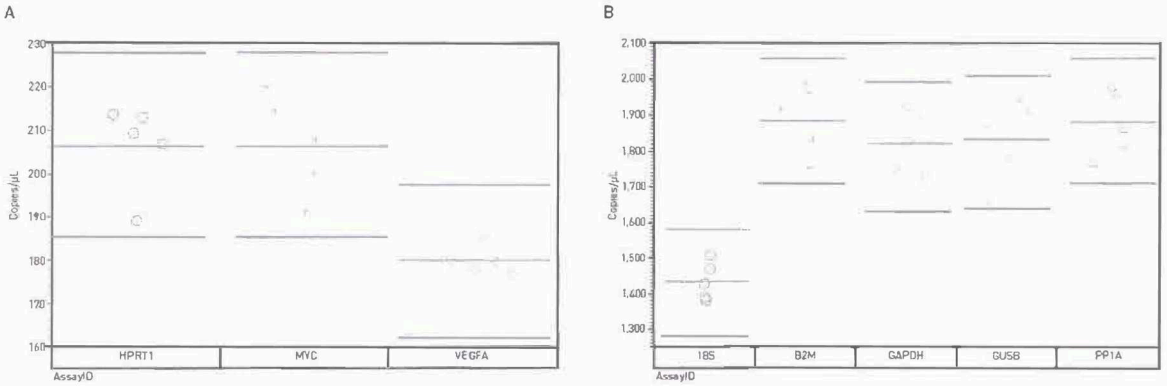

Figure 5. QuantStudio™ 3D Digital PCR System precision. Five independent technical replicates were generated across an 8-assay panel of TaqMan® Gene Expression Assays. All measurements were within ±10% (red lines) of the respective means (green lines). (A) Results for low expressors—HPRT1, MYC, and VEGFA. (B) Results for higher expressors—18S, B2M, GAPDH, GUSB, and PPIA.

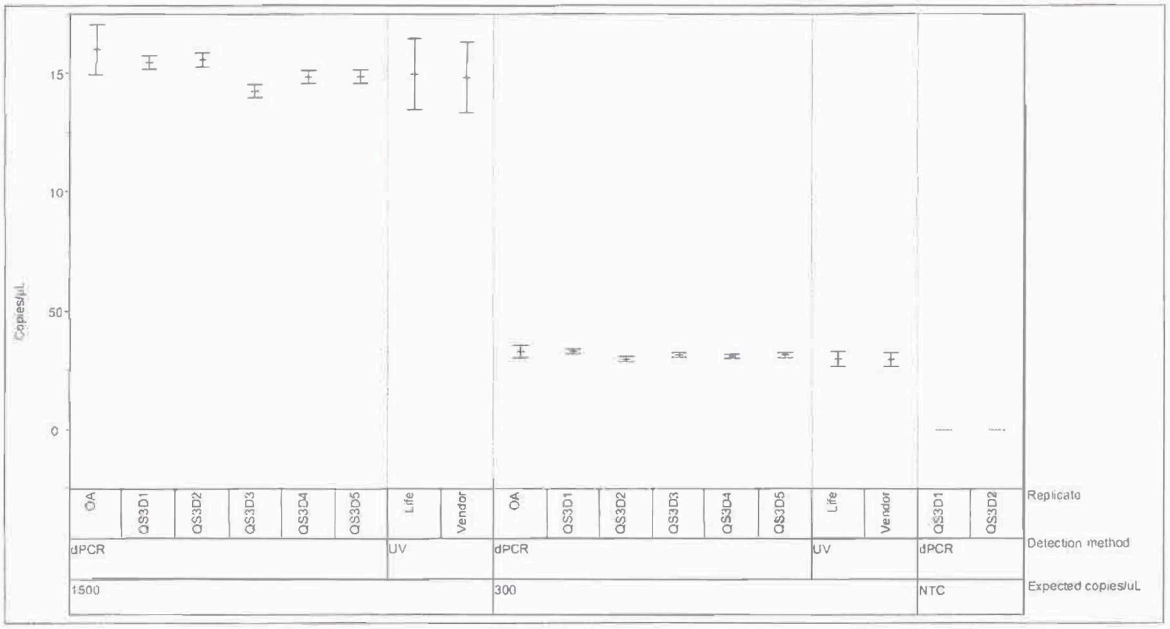

Figure 6. Accuracy on the QuantStudio™ 3D Digital PCR System. The accuracy of absolute quantification on the QuantStudio™ 3D Digital PCR System was assessed by agreement with two orthogonal quantification methods. Using male genomic DNA (Promega), the number of copies/µL at two concentrations was measured and compared using UV spectrophotometer (measured both by Promega (Vendor) and at the Life Technologies™ facility), and digital PCR on the QuantStudio™ 12K Flex OpenArray™ Digital PCR Plates (OA) and 5 replicates on the QuantStudio™ 3D Digital PCR System (QS3D). The expected target concentrations noted by the vendor were 300 copies/µL and 1,500 copies/µL for the two samples. Each method falls within the range of the vendor's expected value, with the measurements from the QuantStudio™ 3D Digital PCR System exhibiting the highest precision relative to the other methods.

Precision and accuracy for copy number analysis

The precision and accuracy of the QuantStudio® 3D system for copy number analysis was measured across a representative panel of nine genomic DNA samples procured from the Coriell repository. Using a standard TaqMan® Copy Number Assay specific to the CCL3L1 genetic locus found on the long arm of chromosome 17, 6 or 8 replicate measurements were made (Figure 7A) indicating that the samples represent copy numbers from 0 to 8 copies per genome. Due to the high precision achievable, a statistically significant difference between the samples containing 7 and 8 copies was clearly discernable, confirming the ability of digital PCR to differentiate less than a 1.2-fold difference (Figure 7B).

A

Sample	Number of replicates	Expected copy number	Detected copy number (mean)	Standard deviation	CV (%)
NA17245	6	0	0.08	0.06	N/A
NA17251	6	1	0.98	0.02	2.21
NA17258	6	2	1.96	0.05	2.47
NA17132	6	3	2.98	0.06	1.85
NA19194	8	4	4.00	0.05	1.22
NA18507	8	5	5.11	0.13	2.50
NA17110	8	6	5.91	0.12	2.07
NA17202	8	7	7.02	0.07	1.02
NA18854	8	8	7.95	0.20	2.55

B

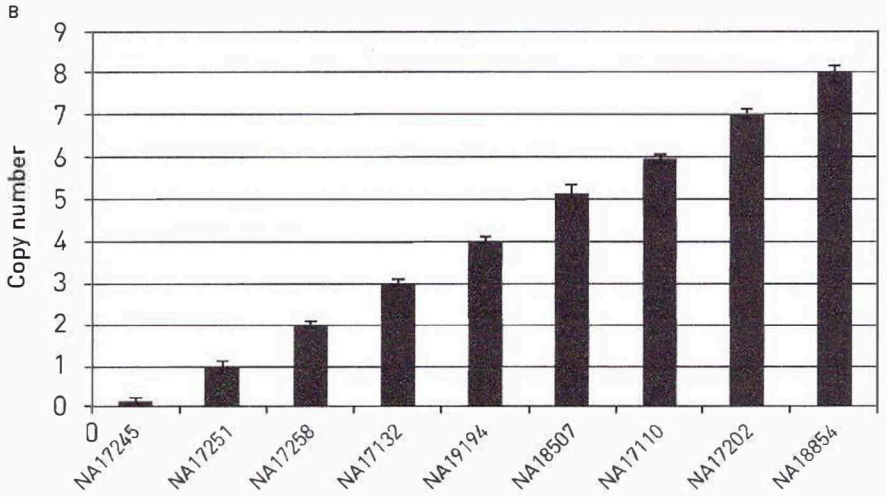

Figure 7. Precision and accuracy demonstrated for copy number analysis of the CCL3L1 genetic locus on chromosome 17 across 9 independent DNA samples representing 0–8 copies. (A) Experimental design and resulting data across the tested sample set. Each digital PCR measurement overlaps the expected value within the standard deviation achieved. It should be noted that all measurement coefficients of variation (CV) were below 2.55%, underscoring the high degree of precision achievable. **(B)** Graphical representation of the same data displayed in A. With the achieved precision, digital PCR results were able to discern the difference between samples containing 7 and 8 copies.

Dynamic range

Digital PCR inherently yields a smaller dynamic range than traditional real-time PCR techniques. Yet with its 20,000 reaction wells, the QuantStudio™ 3D Digital PCR 20K Chip achieves a theoretical 5 logs of dynamic range, enabling a broad number of key digital PCR applications.

Sensitivity

For some research areas, such as oncology, detection of rare genetic changes in cells among a large population of wild type cells poses a significant challenge. Detection of such rare mutations requires high assay sensitivity to distinguish the mutation relative to the wild type background, a capability enabled by digital PCR using standard TaqMan® SNP Genotyping Assays. By subdividing a sample into thousands of individual PCR replicates, the total number of molecules in any given reaction is greatly reduced, effectively enriching for the sequences of interest and diluting out the wild type background. Such mutant molecules may not only be rare relative to the wild type population, but may also be rare in an absolute sense. The ability to amplify single molecules, critical to the concept of digital PCR, enables detection of targets present down to 30 copies and less (Figure 8).

High performance required for your applications

The QuantStudio™ 3D Digital PCR System offers the sensitivity and precision needed for applications such as rare target detection, pathogen and GMO detection, CNV analysis, and quantification of standards. Featuring simple, robust operation and sealed chip design, the QuantStudio™ 3D Digital PCR System can bring high-performance quantification within reach of most labs.

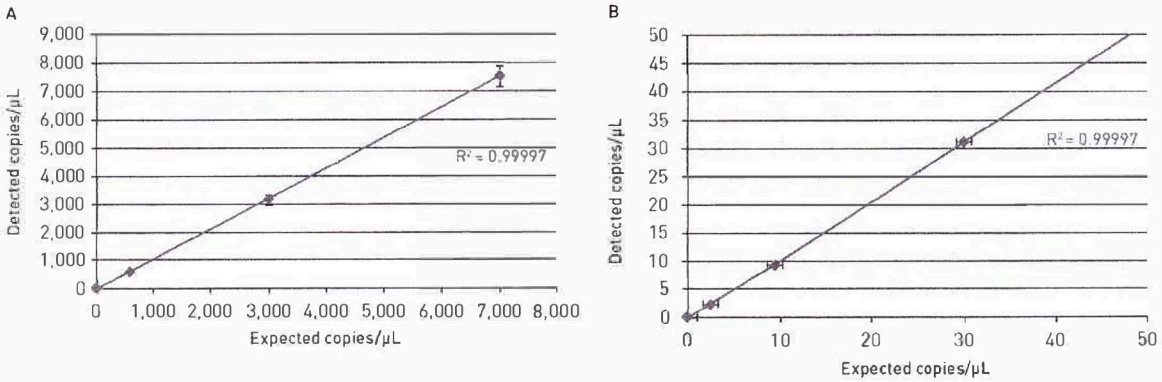

Figure 8. The QuantStudio™ 3D Digital PCR System detects down to less than 30 molecules. (A) Genomic DNA was quantified by UV spectrophotometry, serially diluted from 7,000 copies/µL down to 2.5 copies/µL, and measured with the QuantStudio™ 3D Digital PCR System. (B) The low end of the range is expanded for easier visualization.

QuantStudio® 3D Digital PCR System specifications

QuantStudio® 3D Digital PCR Instrument

Time to read 1 sample	~30 seconds
PCR detection method	Endpoint
Sample illumination	LED
Sample detection	CMOS
Detection channels	FAM™/SYBR®, VIC®, ROX™
Reader size H x W x D	8.3 x 5.3 x 9.15 in. / 21 x 13.5 x 23.25 cm
Weight	5.3 lb / 2.4 kg

QuantStudio® 3D Digital PCR 20K Chip

Partitions	Chip reaction wells
Samples per chip	1
Targets per chip	2
Chip capacity in thermal cycler	24
Reaction wells per sample	20,000
Loading volume	14.5 µL
Sealed workflow	Yes

Performance

Dynamic range	5 logs
Precision at 95% confidence interval	±10%
Chemistry compatability	TaqMan® and SYBR® Green chemistries

Ordering information

Product		Cat. No.
QuantStudio® 3D Digital PCR System Package—includes:*		A25581**
QuantStudio™ 3D Digital PCR Instrument	1 instrument	4489084
QuantStudio® 3D Digital PCR Chip Loader	1 loader	4482592
ProFlex™ 2 x Flat PCR System	1 thermal cycler	4484078
QuantStudio® 3D Digital PCR Chip Adapter Kit	1 kit	4485513
QuantStudio® 3D Digital PCR UV Sealing Kit	1 kit	4488475
QuantStudio® 3D Digital PCR 20K Chip Pack (includes consumables)	12 chips per pack (package includes 8 packs)	4485507
QuantStudio® 3D Digital PCR Master Mix	1.5 mL (package includes 1 tube)	4482710
QuantStudio® 3D Digital PCR Tilt Base for ProFlex™ Thermal Cycler		A24898
Additional items		
QuantStudio® 3D Digital PCR Master Mix	5 mL	4485718
QuantStudio® 3D AnalysisSuite™ Server	1 server system	4489085

*Part numbers listed in bundle are for individual components.
**Cat. No. A25581 is for all regions, except Europe, the Middle East, and Africa (EMEA). Please use Cat. No. A25606 for customers residing in EMEA. Package components are slightly different. Please check with your regional sales representative for details.