

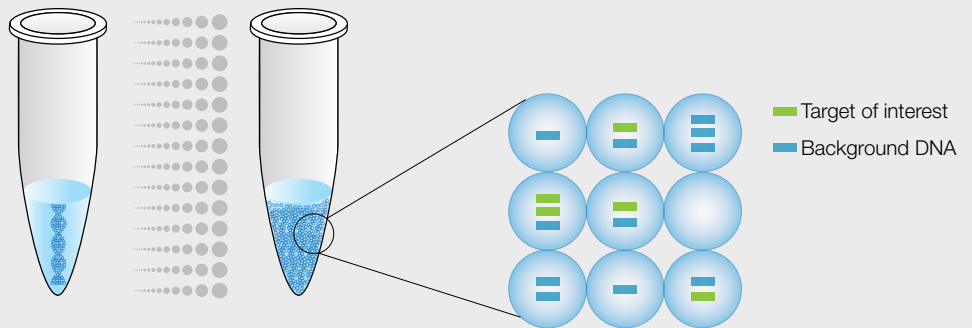
Droplet Digital PCR: QX100 System



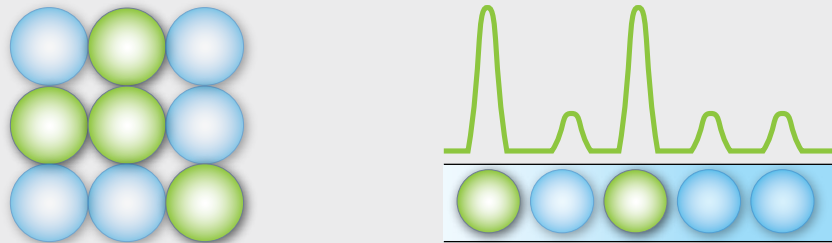
QX100™ Droplet Digital™ PCR System

BIO-RAD

Droplet Digital PCR



The sample is partitioned into 20,000 droplets, with target and background DNA randomly distributed among the droplets.



After PCR amplification, each droplet provides a fluorescent positive or negative signal indicating the target DNA was present or not present after partitioning. Each droplet provides an independent digital measurement.

“X” target copies

Positive and negative droplets are counted for the sample and the software calculates the concentration of target DNA as copies per microliter.



A Breakthrough in Quantitative PCR

The QX100 Droplet Digital PCR system is the third generation of PCR technology. Droplet Digital PCR (ddPCR™) provides an absolute measure of target DNA molecules with unrivaled accuracy, precision, and sensitivity. Applications include copy number variation, rare sequence detection, mutation detection, and gene expression analysis. The QX100 ddPCR system provides single-copy PCR resolution to accelerate discoveries and new strategies for the research of inherited disorders, cancer, and infectious disease.

The QX100 ddPCR system lets you:

- Enrich for rare target sequences
- Detect small differences in target concentration
- Determine copy number without a standard curve

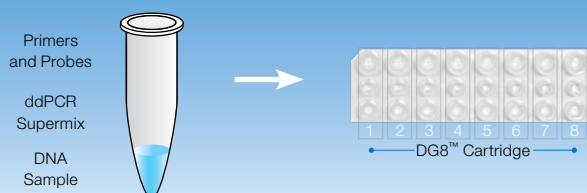


A Simple, Elegant Workflow

0

Prepare PCR-Ready Samples

Combine your DNA sample and primers and probes with the Bio-Rad ddPCR supermix to create eight prepared samples. Load 20 μ l of your prepared samples into individual wells of the eight-channel disposable droplet generator cartridge.



1

Make Droplets

Load the cartridge into the QX100 droplet generator to create an emulsion of ~20,000 monodispersed droplets ready for PCR for each of the eight prepared samples.



Advancing Digital PCR Together

Bio-Rad is applying the precision, accuracy, and sensitivity of Droplet Digital PCR to a range of applications, including copy number variation (CNV) and rare event detection.

Determination of CNV and rare event detection showcases the power that sample partitioning brings to digital PCR. For CNV, the large number of replicates made possible by sample partitioning provides the precision necessary to resolve high-order copy number states. For rare event detection, partitioning increases sensitivity by isolating the target signal from competing background.

Copy Number Variation

Copy number variants include deletions, insertions, duplications, and complex amplifications. Like single nucleotide polymorphisms (SNPs), they are widespread throughout the genome and are associated with human disease.

Analyzing and cataloging CNVs has become an important goal. Progress has been made identifying CNVs using arrays and deep sequencing methods, but validation options are severely limited. The QX100 ddPCR system, which measures copy number with the highest accuracy and precision available, offers an ideal solution for CNV validation at the throughput and running costs necessary to meet this challenge.

2

Perform PCR

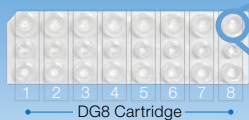
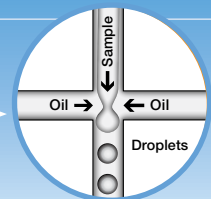
Pipet emulsified samples from the cartridge to a 96-well PCR plate. Perform PCR to end point (40 cycles) using a thermal cycler.



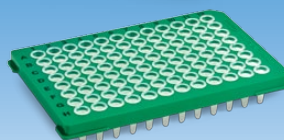
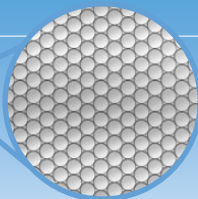
QX100 Droplet Generator



C1000 Touch™ Thermal Cycler

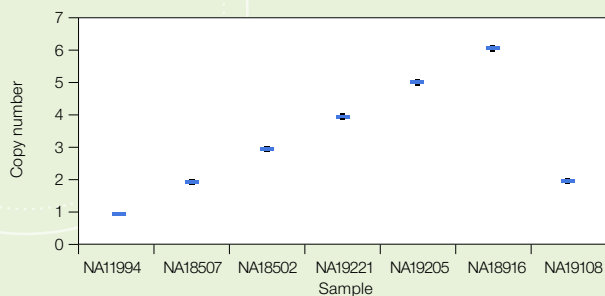


DG8 Cartridge

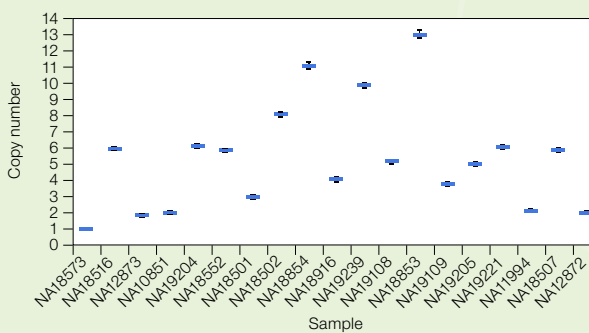


Copy Number Variation Data

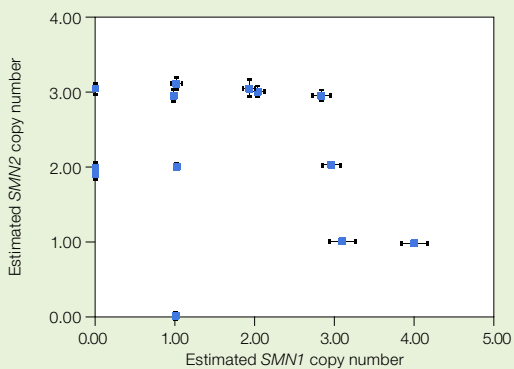
A. CNV Analysis of *MRGPRX1*



C. CNV Analysis of *CCL3L1*



B. CNV Analysis of *SMN1* and *SMN2**



Calculation of copy number variation. **A**, for *MRGPRX1*, the copy number states from 1 to 6 were completely resolved. **B**, *SMN1* and *SMN2* copy number estimation is plotted in two dimensions where each point represents an individual sample. The allelic discrimination 5' nuclease assays for *SMN1* and *SMN2* allow researchers to count genes that differ by only one nucleotide. **C**, *CCL3L1* gene copy number determination on 19 samples demonstrates the accuracy and precision of ddPCR. The data demonstrate that copy number measurements cluster on integers up to 13 copies. Results validate previous next-generation genome sequencing data, indicating 5.7 copies for NA18507.** Samples were obtained from the Coriell Institute. All error bars represent 95% Poisson confidence intervals (CIs).

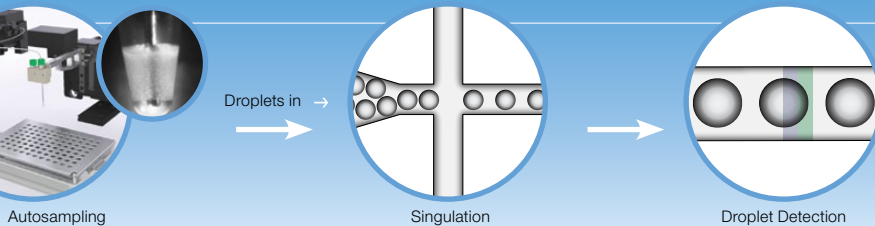
* Replicate variation (95% CI).

** Alkan C et al. (2009). Nat Gen 41, 1061–1067.

3

Read Droplets

Load the plate into the QX100 droplet reader and start your run. Each well is read serially. Droplets are sipped and the singulator unpacks the emulsified droplets and streams them in single file past a two-color optical detection system to determine which droplets contain a target (+) and which do not (-).



4

Analyze Results

ddPCR software reads the positive and negative droplets in each sample and plots the fluorescence droplet by droplet: ~1.4 million droplets are read per 96-well plate. The fraction of positive droplets determines the concentration of the target in the sample.



QX100 Droplet Reader

Rare Event Detection

Rare event detection includes single nucleotide mutation detection, alteration of copy number, and deletion or insertion of nucleotides. Rare event detection has become an important tool in the fight against cancer.

Alteration of Copy Number

Droplet Digital PCR offers a practical solution for the precise estimate of DNA copy number. It can detect amplifications even in highly heterogeneous matrices where only a fraction of the cells are affected. This

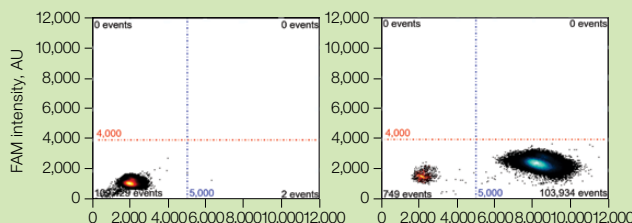
precision enables the detection of somatic copy number alteration — the hallmark of many cancers.

Mutation Detection

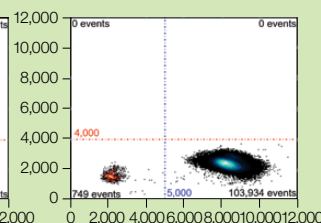
The detection of point mutations requires a high degree of sensitivity, which is available with ddPCR, enabling detection of 0.001% mutant fractions. Measuring extremely low levels of mutant could lead to dramatically more sensitive and less invasive diagnostics.

Mutation Detection Data

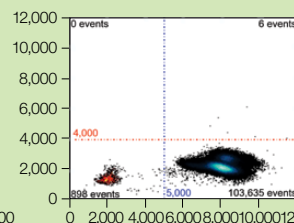
No Template Control



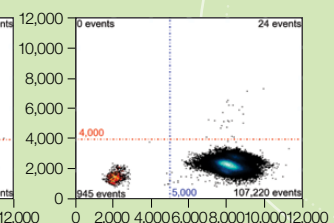
0% Mutant (wild type only)



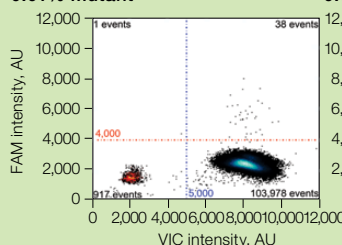
0.001% Mutant



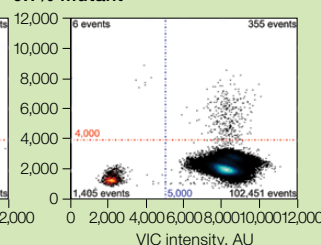
0.005% Mutant



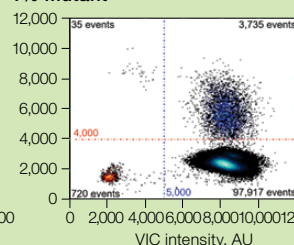
0.01% Mutant



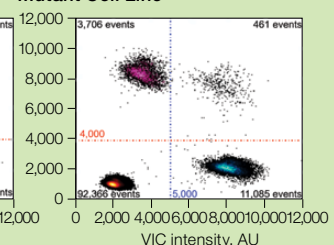
0.1% Mutant



1% Mutant



Mutant Cell Line

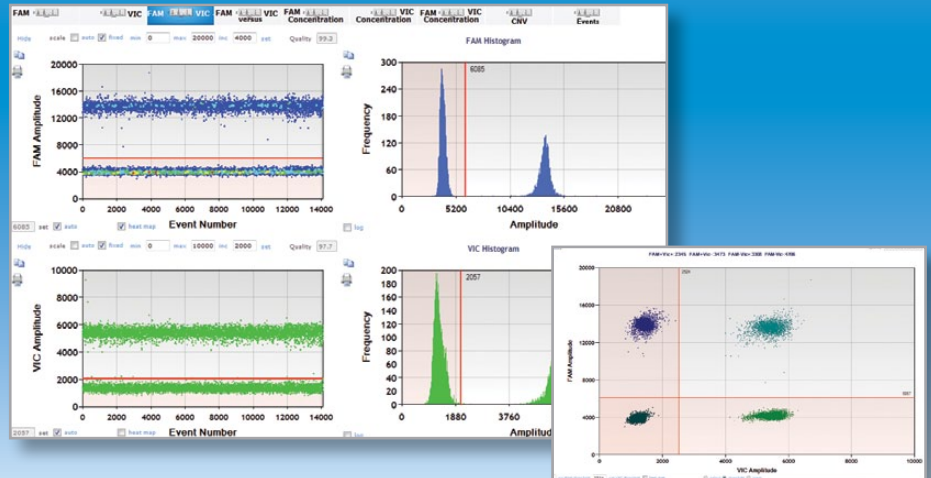


Duplex PCR using TaqMan probes targeting the *BRAF* V600E mutation. The data show ddPCR detects a 0.001% mutant fraction. AU, absorbance units.

5

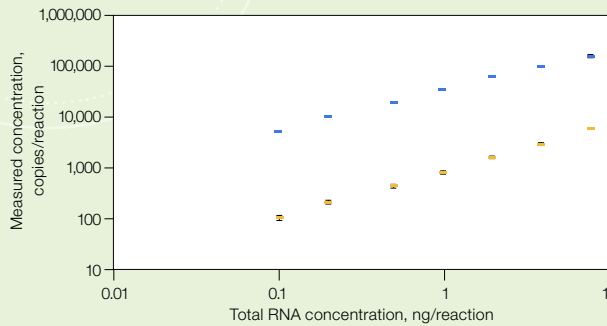
Visualize Data

ddPCR software allows you to visualize the data in a variety of ways and determine concentrations in copies/μl.



Gene Expression Data

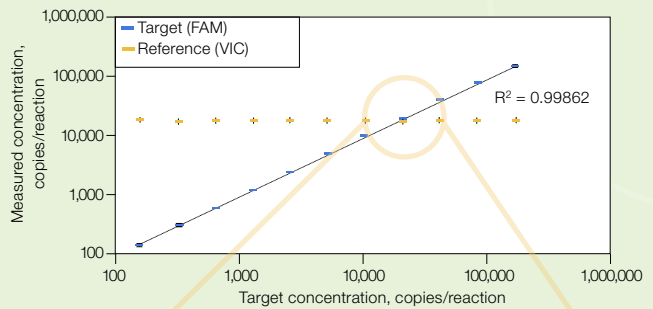
Droplet Digital PCR enables gene expression analysis with $\pm 10\%$ precision.



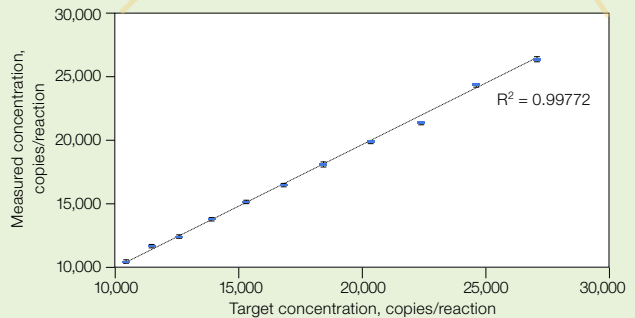
The data show a dilution series of two mRNAs. β -actin (■); ANP32B (■).

Performance Data

A



B



Dynamic range. A, concentration results for twofold dilutions from 150 to 150,000 copies/reaction; B, concentration results for 1.1-fold dilutions demonstrate precision at $\pm 10\%$ with a 95% CI. The error bars are at the 95% CI. The reference gene (■) was constant at 19,000 copies/reaction.

Specifications

QX100 Droplet Generator

Starting sample size	20 µl
Capacity	8 samples/cartridge
Droplets per sample	20,000
Dimensions (W x D x H)	28 x 36 x 13 cm (11 x 14 x 5")
Weight	4.5 kg (10 lb)

QX100 Droplet Reader

Throughput	32 wells/hr
Sample illumination	Light-emitting diodes
Sample detection	Photomultiplier tubes
Detection channels	FAM, HEX (VIC)
Dimensions (W x D x H)	66 x 52 x 29 cm (26 x 20 x 11")
Weight	26 kg (57 lb)
Linear dynamic range	5 orders of magnitude
Precision	±10%
Droplets per 96-well plate	1.4 million

Catalog

Description

Kits and Reagents for QX100 Droplet Digital PCR System

186-3010	ddPCR Supermix for Probes , 5 ml (5 x 1 ml), 2x supermix, for use in sample preparation for droplet generator in the QX100 Droplet Digital PCR system
186-3024	Droplet PCR Supermix , 5 ml (5 x 1 ml), 2x supermix, for use in sample preparation for droplet generator in the QX100 Droplet Digital PCR system
186-3021	One-Step RT-ddPCR Kit for Probes , 2 ml (2 x 1 ml), 200 x 20 µl reactions, 2x RT-ddPCR mix, includes 1 manganese acetate tube, for use with QX100 Droplet Digital PCR system
186-3022	One-Step RT-ddPCR Kit for Probes , 5 ml (5 x 1 ml), 500 x 20 µl reactions, 2x RT-ddPCR mix, includes 2 manganese acetate tubes, for use with QX100 Droplet Digital PCR system
186-3052	ddPCR Buffer Control Kit , 9 ml (2 x 4.5 ml), 2x buffer, for use as a blank well control in QX100 Droplet Digital PCR system

Ordering Information

Catalog #	Description
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QX100 Droplet Digital PCR System

186-3001	QX100 Droplet Digital PCR System , includes droplet generator, droplet reader, laptop computer, software, associated component consumables
186-3002	QX100 Droplet Generator , for use with QX100 Droplet Digital PCR system
186-3003	QX100 Droplet Reader , for use with QX100 Droplet Digital PCR system

Accessories for QX100 Droplet Digital PCR System

186-3006	Droplet Generator Cartridges and Gaskets , includes 5 packages of 1 x 24 DG8 cartridges, 5 packages of 1 x 24 DG8 gaskets
186-3008	DG8 Cartridges for QX100 Droplet Generator , 1 package of 24 cartridges
186-3009	DG8 Gaskets for QX100 Droplet Generator , 1 package of 24 gaskets
185-1196	C1000 Touch Thermal Cycler with 96-Well Fast Reaction Module , includes C1000 Touch thermal cycler chassis, 96-well fast reaction module, USB flash drive
185-1197	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module , includes C1000 Touch thermal cycler chassis, 96-deep well reaction module, USB flash drive
185-2197	S1000™ Thermal Cycler with 96-Deep Well Reaction Module , includes S1000 thermal cycler chassis, 96-deep well reaction module

Reference

Alkan C et al. (2009). Personalized copy number and segmental duplication maps using next-generation sequencing. *Nat Genet* 41, 1061–1067.

FAM and VIC are trademarks of Applied Biosystems. TaqMan is a trademark of Roche Molecular Systems, Inc.

Bio-Rad's real-time thermal cyclers are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Numbers 6,767,512 and 7,074,367.



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