Assays for Droplet Digital PCR

BIO-RAD

WORKFLOW

A VERSATILE AND SCALABLE WORKFLOW

Bio-Rad's Droplet Digital PCR (ddPCR) technology is based on water-oil emulsion droplet technology to partition the sample. The ddPCR technology is composed of a droplet generator, a droplet reader, and associated consumables. The droplet generator partitions each sample into 20,000 uniform nanoliter-sized droplets containing target and background DNA in random distribution. Each droplet contains a separate reaction. Droplets are transferred to a 96-well PCR plate and PCR is performed to end point in a thermal cycler. The droplets are singulated and pass by an optical detection system. Up to 96 samples can be processed per run.

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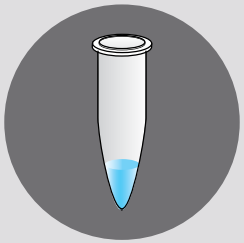

Prepare ddPCR reaction mix

- Combine DNA/RNA sample, primers, and/or probes with one of Bio-Rad's ddPCR supermixes
- Fully validated ddPCR assays can be used

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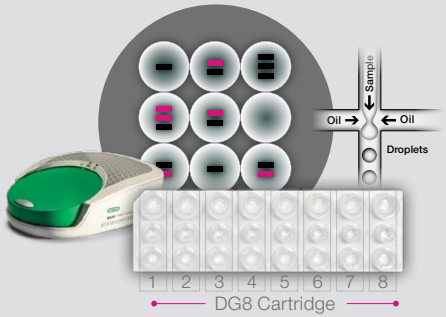
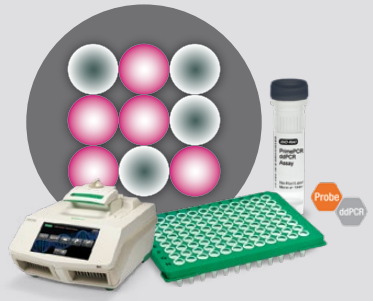


Generate droplets

- Load the ddPCR reaction mix into the wells of a droplet generator cartridge
- 8 x 20,000 droplets are generated from each run in the QX200 Droplet Generator
- Target DNA (▬) and background DNA (▬) are randomly distributed in droplets

3



Perform PCR

- Transfer the droplets to a 96-well PCR plate and seal the plate
- Run the PCR protocol

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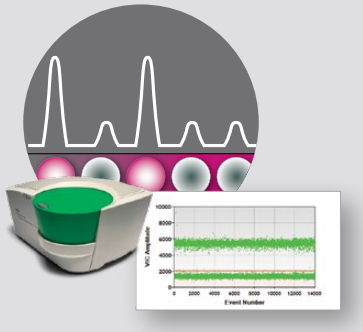
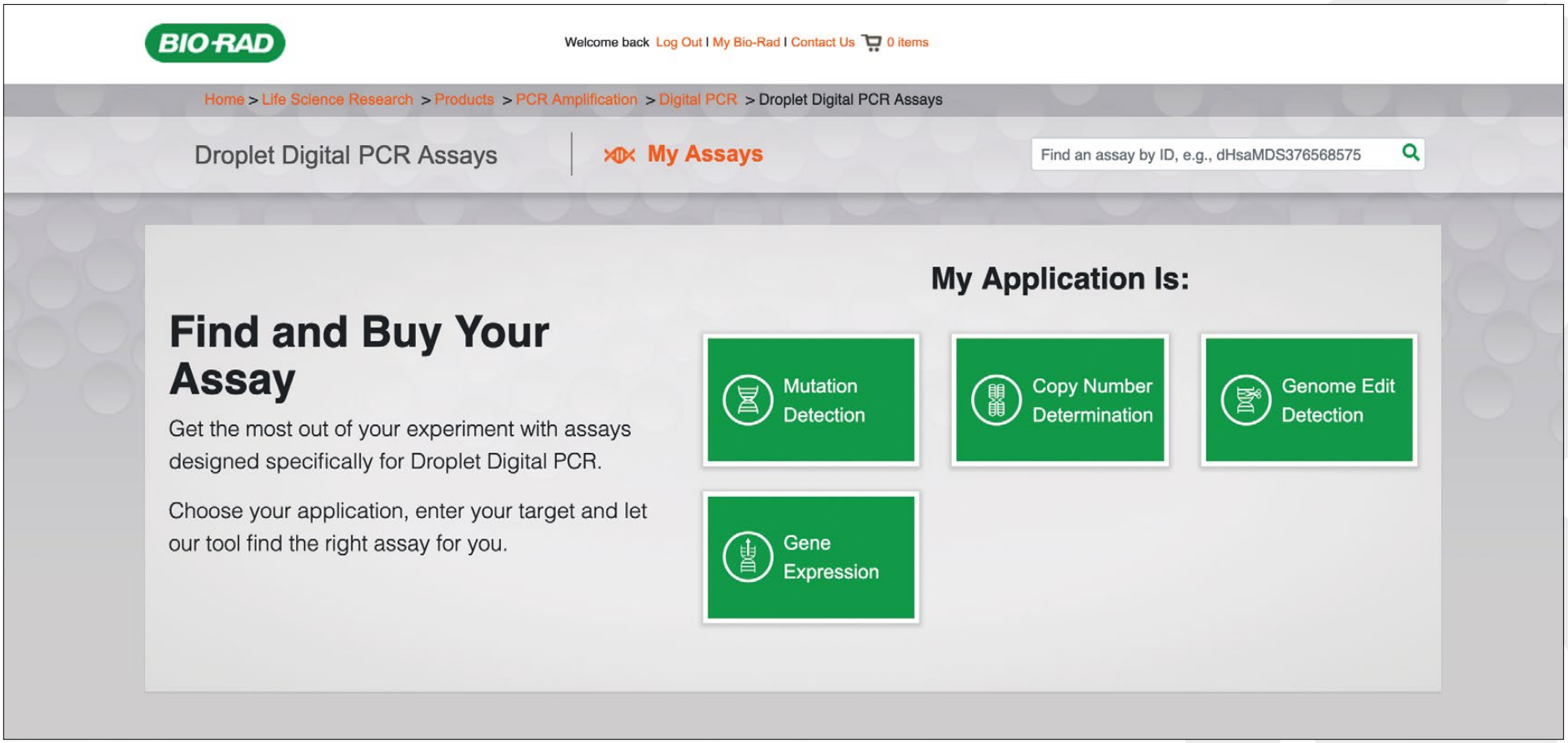


Read and analyze results

- After PCR, load the 96-well PCR plate into the QX200 Droplet Reader
- Positive and negative droplets in each sample are read
- Analyze concentrations

EXPERTLY DESIGNED ASSAYS ARE A CLICK AWAY THROUGH OUR ASSAY DESIGN ENGINE

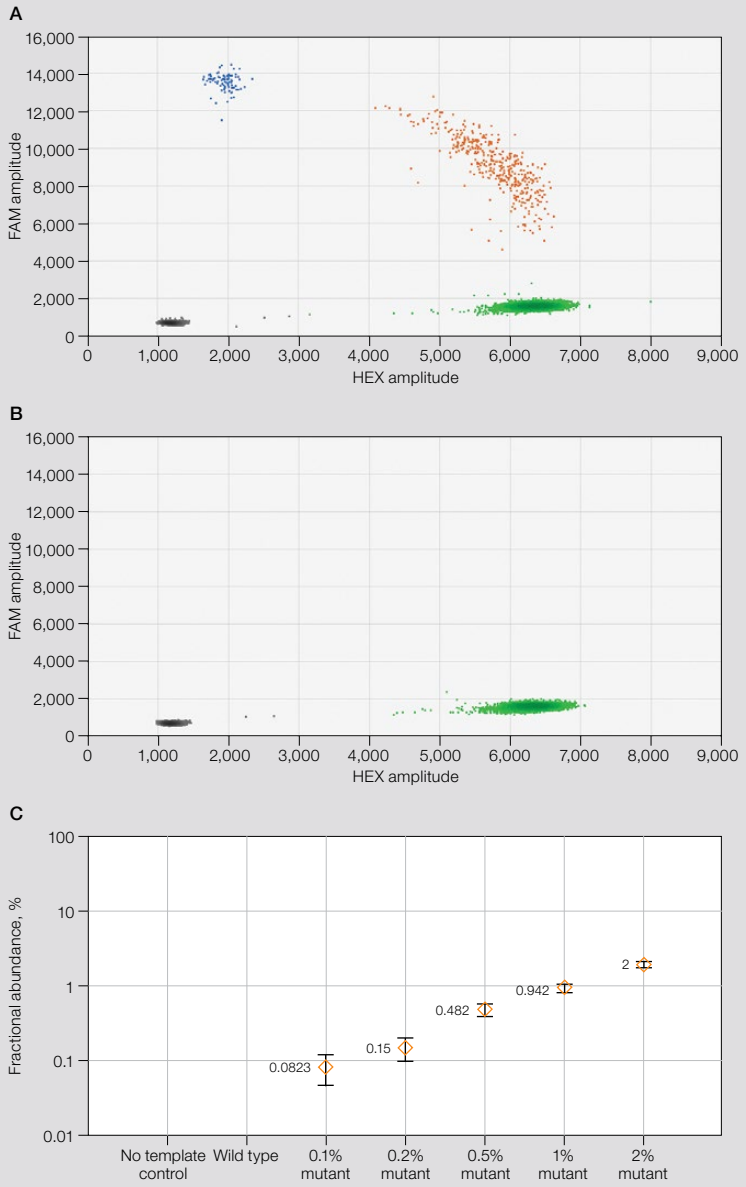
Go to **bio-rad.com/Digital-Assays** for more information.



MUTATION DETECTION ASSAYS

THE NEW GOLD STANDARD IN QUANTIFYING RARE CANCER MUTATIONS

One application that harnesses the power of ddPCR is mutation detection, where a biomarker exists within a background of a highly abundant counterpart that can differ by only a single nucleotide. Many methods for mutation analysis have poor selectivity and fail to detect mutant sequences with abundances of less than one in 100 wild-type sequences. Mutation detection assays on the ddPCR platform enable detection of minute mutant fractions without pre-amp because partitioning increases sensitivity by isolating the target signal from competing background.

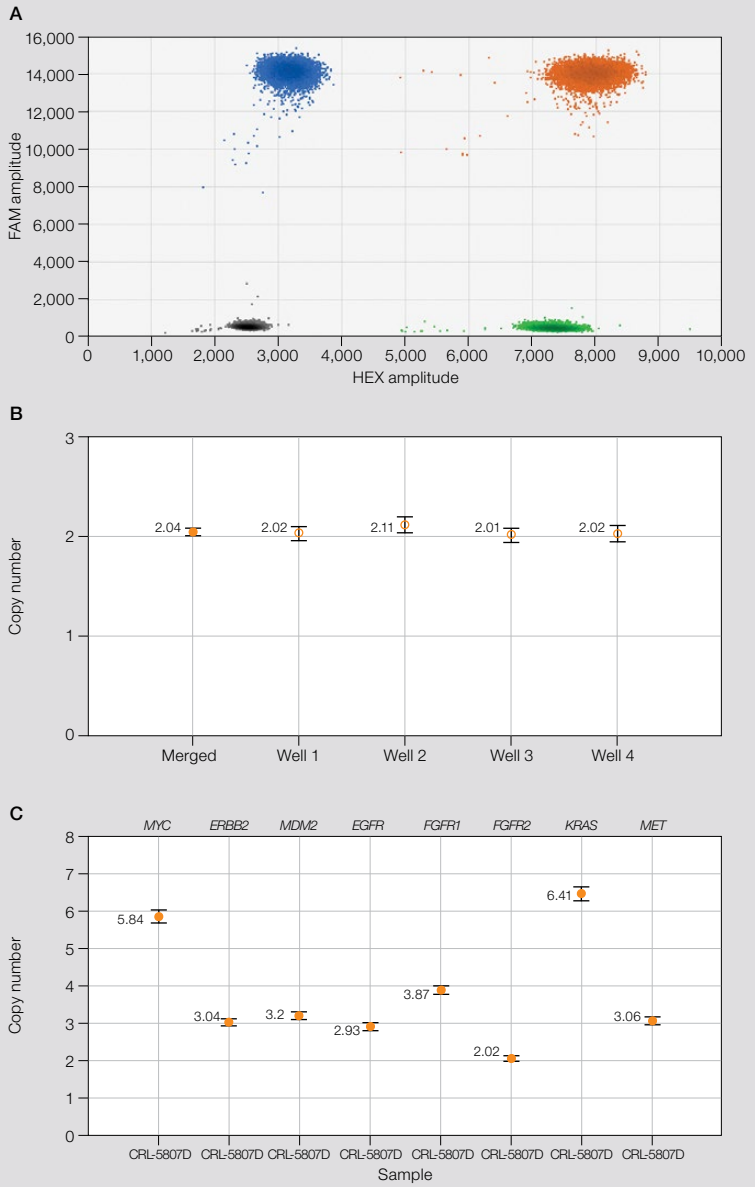


High sensitivity of ddPCR mutation detection assays provides precise quantification of *JAK2 V617F* mutation in the presence of wild-type DNA. A, 2-D fluorescence amplitude plot shows duplicate wells of a mixed mutant: wild-type sample across multiple percentage mutant blends. The black cluster on the plot represents the negative droplets, the green cluster represents the droplets that are positive for wild-type DNA only, the blue cluster represents the droplets that are positive for mutant DNA only, and the orange cluster represents the droplets that are positive for both targets. **B**, 2-D fluorescence amplitude plot shows duplicate wells of a wild-type–only sample. **C**, fractional abundance plot shows the percentage frequency (orange markers) of the mutant DNA in a wild-type DNA background. All error bars represent the 95% confidence interval.

COPY NUMBER ASSAYS

AT LAST — IDENTIFYING COPY NUMBER VARIATIONS WITH CONFIDENCE

The major technical challenge in copy number assessment is the ability to discriminate, with statistical confidence, between consecutive copy number states. Current methods to analyze copy number variation (CNV) include array comparative genomic hybridization (aCGH), quantitative PCR (qPCR), and sequencing. These methods lack the sensitivity and resolution needed for this fine degree of quantitative discrimination in CNV analysis. The massive partitioning of a ddPCR reaction into 20,000 droplets enables the discrimination required to resolve small fold changes.



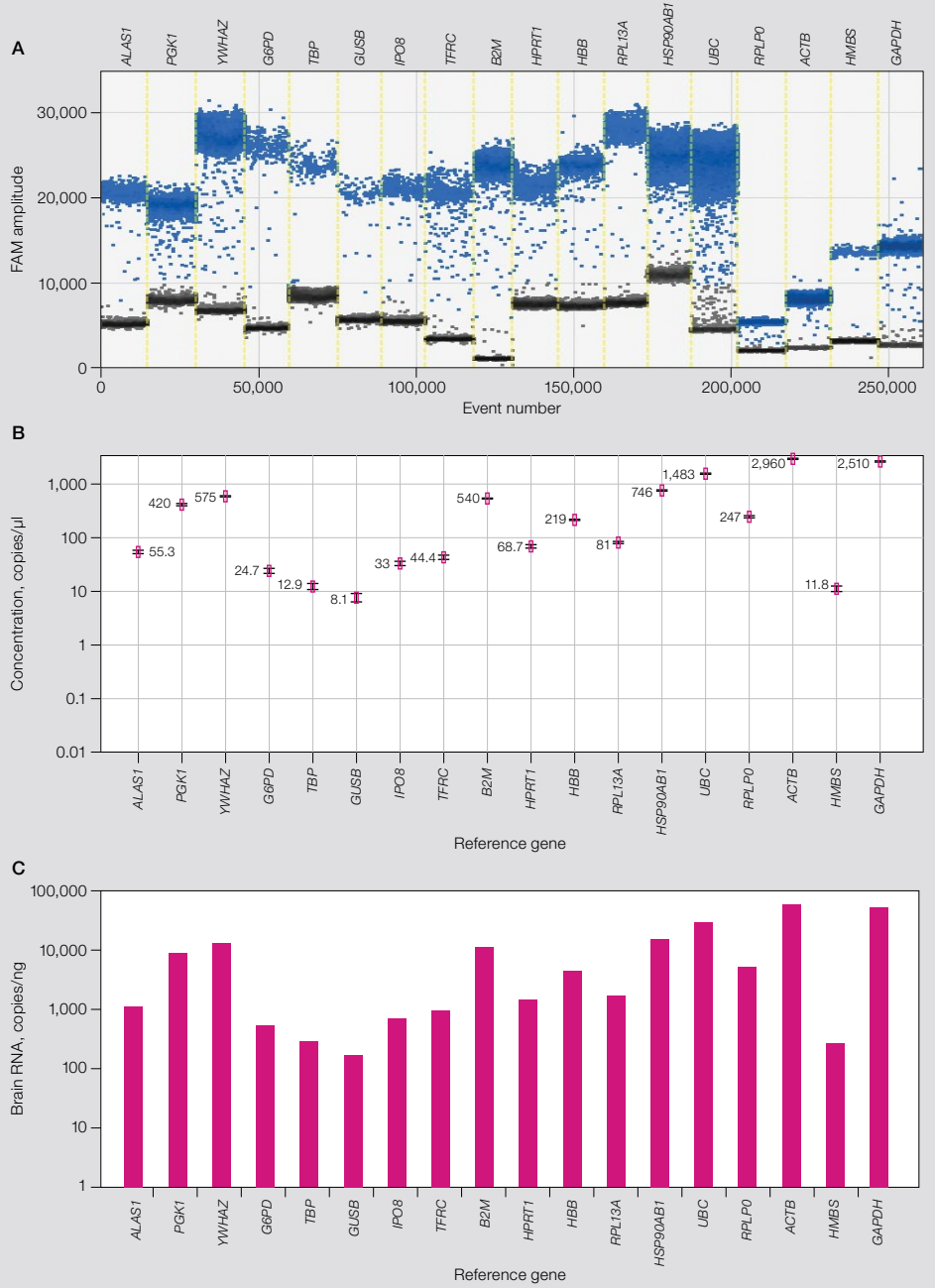
Droplet Digital PCR copy number assays provide superior resolution and precision. **A**, 2-D fluorescence amplitude plot shows four replicate wells of a copy number sample duplexed with *ERBB2* and *RPP30* assays. The black cluster on the plot represents the negative droplets, the green cluster represents the droplets that are positive for *RPP30* reference only, the blue cluster represents the droplets that are positive for *ERBB2* only, and the orange cluster represents the droplets that are positive for both *ERBB2* and *RPP30* targets. **B**, copy number plot shows multiple wells at copy number 2 with precise replicate and merged well values. **C**, copy number plot shows multiple oncogene copy number determinations for various genes with the same NCI-H358 lung cancer sample. All error bars represent the 95% confidence interval.

GENE EXPRESSION ASSAYS

BETTER GENE EXPRESSION DATA FOR SIMILAR COST

With its high precision, ddPCR can be used to detect small fold changes in expression of a target gene between samples. Choose from over 40,000 existing human and mouse gene expression assays, which can be used with QX200 ddPCR EvaGreen® Supermix and modified thermal cycling conditions.

- Contrary to expectations, multiplexed ddPCR gene expression studies have similar costs to singleplex SYBR® qPCR studies
- Singlicate multiplexed ddPCR studies make much better use of rare samples than SYBR® qPCR
- Droplet Digital PCR allows advanced new strategies such as SELFIE Digital PCR



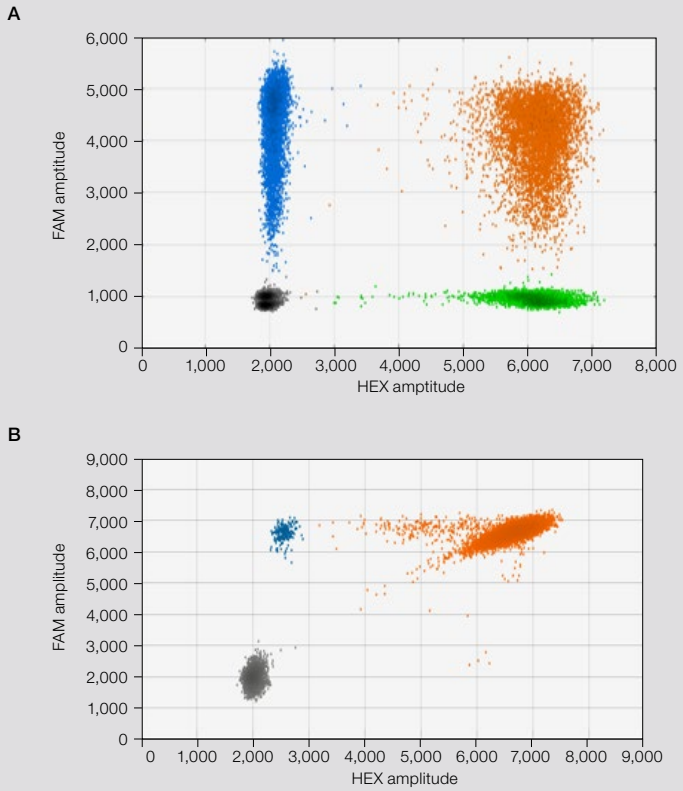
Robust gene expression assays used in ddPCR with EvaGreen® provide precise quantification of 18 reference genes. A, 1-D fluorescence amplitude plot shows results of a two-step reverse transcription ddPCR reference assay panel run on human brain RNA. The black clusters on the plot represent the negative droplets and the blue clusters represent the droplets that are positive for the respective reference assay. **B**, concentration plot shows various gene concentrations of cDNA calculated as copies/µl on a log10 scale. **C**, calculated RNA concentrations in the original human brain RNA sample for each reference gene. All error bars represent the 95% confidence interval.

GENOME EDIT DETECTION ASSAYS

FAST. SIMPLE. PRECISE.

Droplet Digital PCR enables a rapid, cost-effective, and ultrasensitive method for detecting genome editing events created using nucleases that cause double-stranded breaks in DNA, such as CRISPR-Cas9 or other gene editing tools.

- Detect HDR (homology directed repair) and NHEJ (non-homologous end joining) events present at frequencies of ≤0.5%
- Provide absolute quantification of genome editing from as little as 5 ng of total gDNA
- Design and order ddPCR Assays to detect HDR and NHEJ edit events for any target on Bio-Rad's Digital Assay Site



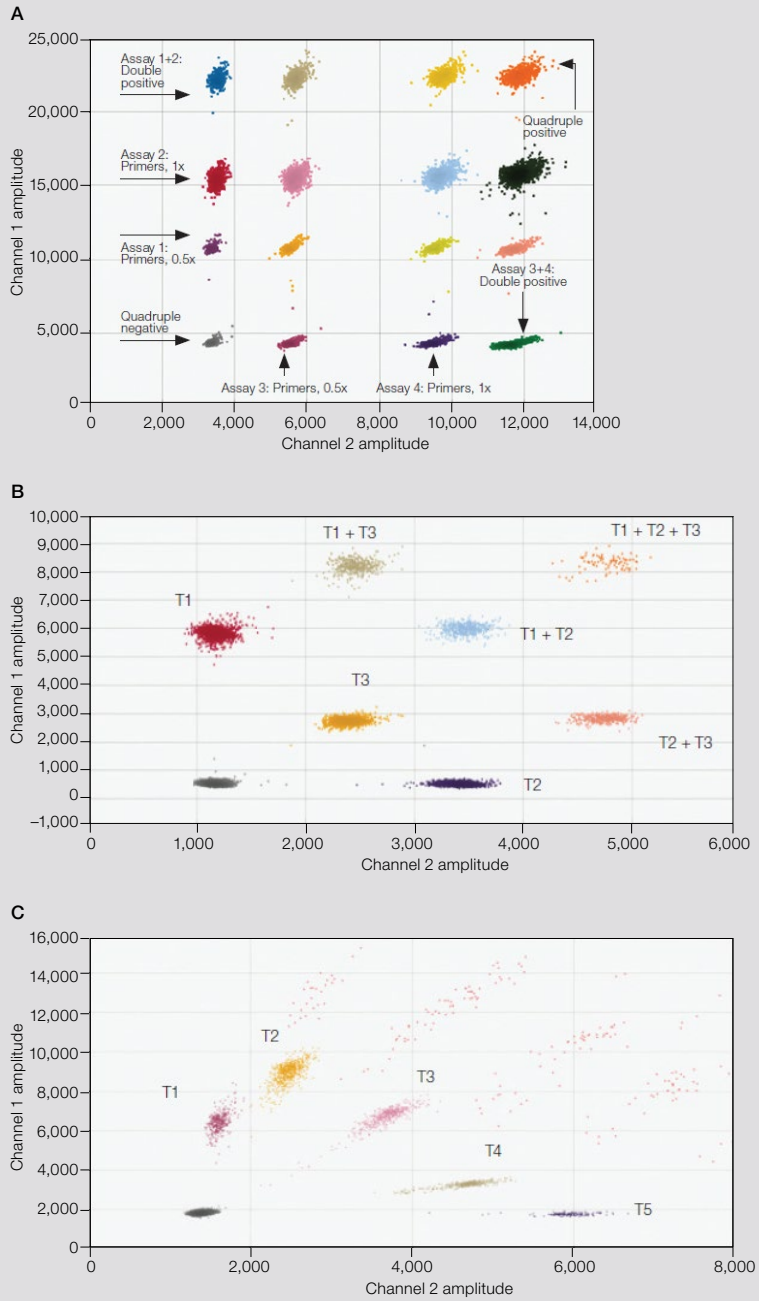
HDR and NHEJ assay readouts. A, HDR mutation-positive control (WT + 20% gblocks gene fragment containing a single base pair substitution) with HDR GED + HDR REF Assay. The HDR edits are FAM+HEX–, while the HEX+ and FAM+HEX+ positive clusters represent the total number of copies, including WT, NHEJ, and HDR edits. **B**, NHEJ mutation-positive control (WT + 1% gblocks gene fragment containing a one base pair deletion at the predicted cut site). The WT cluster is positive for both FAM and HEX, while the NHEJ mutant single-positive droplets are positive for FAM only.

MULTIPLEXING STRATEGIES

HIGHER-ORDER MULTIPLEXING — SAME COLOR CHANNELS, MORE ANSWERS

The number of targets measured in an assay need not be limited to the number of color channels available on Bio-Rad's Droplet Digital PCR Systems. Multiple targets can be measured in a single reaction, enabling higher-order multiplexing. Various strategies can be employed to maximize data generated from each channel:

- **Amplitude-Based Multiplexing** — targets are detected via probes conjugated with a single dye at different final concentrations in an endpoint reaction
- **Radial/Ratio-Based Multiplexing** — targets are detected using probes mixed at different probe ratios to create unique endpoint fluorescence.
- **Nondiscriminating Multiplexing** — targets are detected but not uniquely identified

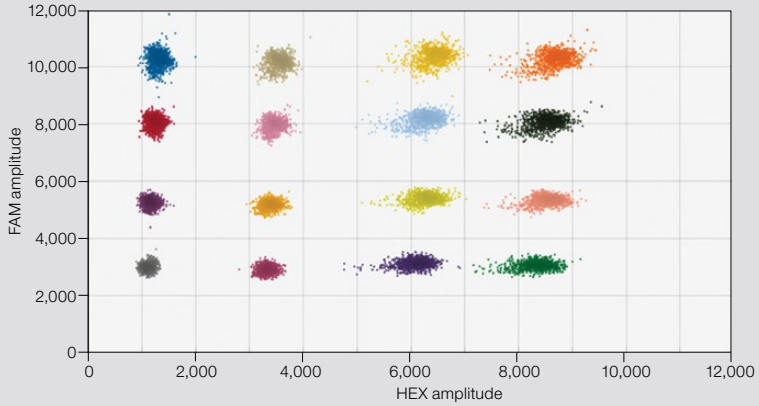


Multiplexing with two color channels: A, detection of four targets by amplitude multiplexing. **B**, detection of three targets by radial/ratio-based multiplexing. Assay used the following ratios of FAM to HEX: T1, 1:0; T2, 0:1; T3, 1:1. **C**, detection of five targets by radial/ratio-based multiplexing. Assay used the following ratios of FAM to HEX: T1, 1:0; T2, 3:1; T3, 1:1; T4, 1:3; T5, 0:1.

ADVANCED MULTIPLEXING — MORE COLOR CHANNELS, EVEN MORE ANSWERS

Bio-Rad's QX ONE Droplet Digital PCR System offers four color channels — FAM, HEX, Cy5, and Cy5.5 — thereby providing additional multiplexing flexibility. By using Bio-Rad's ddPCR Multiplex Supermix on the QX ONE ddPCR System, as many as eight targets can be detected and measured in a single reaction. Such advanced multiplexing is made possible using strategies such as amplitude multiplexing in conjunction with four color channels. Extract as much information as possible with high sensitivity, using as little sample as possible in a fast, cost-effective manner.

A



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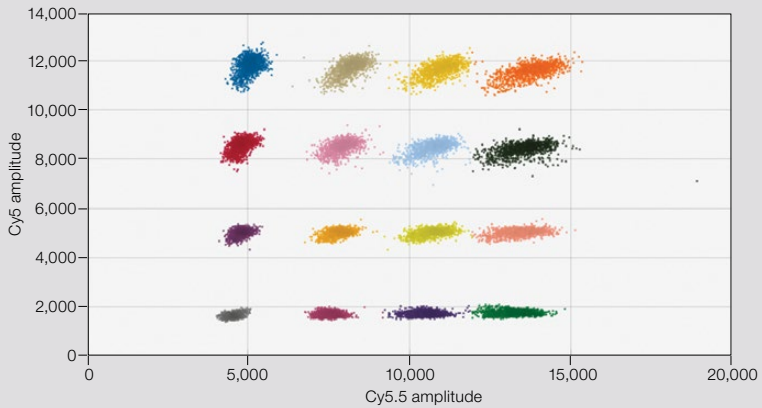

C

Dye Name	Target	Concentration, copies/µl	CNV
FAM Lo	*PLAU*	668	1.96
FAM Hi	*CCND1*	658	1.95
HEX Lo	*VCL*	662	1.96
HEX Hi	*REN*	665	1.97
Cy5 Lo	*BRCA1*	658	1.95
Cy5 Hi	*KLF8*	691	2.05
Cy5.5 Lo	*SORL1*	672	1.99
Cy5.5 Hi	*PTEN*	675	—

8-plex amplitude multiplexing on QX ONE System. A, detection of four targets on FAM/HEX 2-D plot. *PLAU* (FAM Lo), *CCND1* (FAM Hi), *VCL* (HEX Lo), *REN* (HEX Hi). **B**, detection of four targets on Cy5/Cy5.5 2-D plot. *BRCA1* (Cy5 Lo), *KLF8* (Cy5 Hi), *SORL1* (Cy5.5 Lo), *PTEN* (Cy5.5 Hi). **C**, measured concentration of each target in copies/µl. *PTEN* (Cy5.5 Hi) is reference.

SOLUTIONS OFFERED FOR NUMEROUS APPLICATIONS

Droplet Digital PCR Assays and Kits are made for numerous applications, including mutation detection, copy number determination, genome edit detection, gene expression, residual DNA quantification, and library quantification.

Multiplex Mutation Screening

For rapid screening of several key cancer mutations in a single reaction.

- High sensitivity (limit of detection ≤ 0.5%)
- Work with low amounts of input DNA without pre-amplification
- Assays available for *BRAF V600*, *KRAS G12/G13*, *KRAS Q61*, *NRAS G12*, *NRAS G12/G13*, *RNAS Q61*, *EGFT* exon 19 deletions

Copy Number

For the detection of *SMN1* and *SMN2* gene copy number.

- Achieve superior performance with accurate and reproducible copy number calls for the gene of interest
- Perform high-throughput screening by processing several hundred samples in a single day

Residual DNA Quantification

For detection of CHO and *E. coli* host cell DNA.

- Highly precise, femtogram-level quantification of residual CHO or *E. coli* DNA
- Direct quantification without DNA purification step

Library Quantification

For the quantification of NGS sequencing libraries.

- Provide information about library quality, such as adapter dimers, and indicate library insert size
- Provide more efficient and consistent loading of libraries for sequencing runs
- Enable balancing of pooled library samples

FLEXIBLE ASSAY DESIGN OPTIONS

Custom

Know your oligo sequences and just want to order it from a trusted source?

bio-rad.com/ddPCRCustomAssays

Assay Design Service

Have a unique assay need? Contact your sales representative and work with a specialist. We'll design it for you.

bio-rad.com/ddPCRAssayDesignService

Expert Design

See a list of our most sought after specialty assays, such as the Microsatellite Instability Assay for detection of MSI markers (*BAT25/BAT26*, *NR21/NR24*, and *Mono27*).

bio-rad.com/ddPCRExpertDesign

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