PCR PRIMERS

PrimePCR™ Probe Assays for Droplet Digital™ PCR

- Predesigned, wet-lab validated assays
- Precise detection of small fold target changes
- Sensitive detection of cancer biomarkers

Copy Number and Mutation Detection Assays

PrimePCR probe assays for Droplet Digital PCR (ddPCR™) enable precise quantification without a standard curve. PrimePCR ddPCR assays are predesigned, wet-lab validated assays and are available in 200, 1,000, and 2,500 reaction sizes in the following formats:

- Mutation detection assays
- Copy number detection assays

Key benefits of the PrimePCR assays for ddPCR include:

- Universal cycling conditions and primer/probe design strategy
- Universal restriction enzyme digestion strategy for copy number assays
- World class design and manufacturing expertise (Digital Biology Center, Biogazelle, and Integrated DNA Technologies)

Droplet Digital PCR Technology

Droplet Digital PCR is based on water-oil emulsion droplet technology to partition the sample. This large-scale sample partitioning allows measurement of thousands of independent amplification events within a single sample. Each droplet contains nucleic acid template and other PCR components and is amplified to end point with probes. Sample concentrations are determined based on the number of fluorescent positive and negative droplets in a sample well.

Mutation Detection Assays

One application that harnesses the power of ddPCR is mutation detection, where a biomarker exists within a background of a highly abundant counterpart that differs 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. Methods with better specificity and sensitivity for somatic mutations are therefore needed.

Mutation detection assays (Figures 1 and 2) on the ddPCR platform enable detection of 0.001% mutant fractions because partitioning increases sensitivity by isolating the target signal from competing background. Measuring extremely low levels of mutants could lead to dramatically more sensitive and less invasive diagnostics.

Assay Specifications

- 12 target assays for wild-type and mutant targets (Table 1)
- 1:2,000 detection of mutant:wild-type ratio in a single well
- Uniform PCR conditions and primer/probe strategy
- Targets are of biological significance and obtained from the catalog of somatic mutations in cancer database (V57)

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Table 1. Wild-type and mutant targets.

Wild-type targets	*BRAF*	*EGFR*	*HRAS*	*KIT*	*KRAS*
Mutant targets	*V600E*	*L858R*	*G12V*	*D816V*	*G12A*
		T790M			*G12C*
					G12D
					G12R
					G12S
					G12V
					G13D

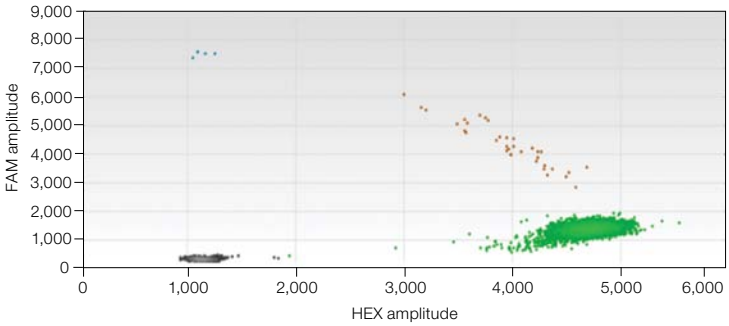

Fig. 1. ddPCR amplitude scatter plot (wild type + mutant). Single-well data for mutant DNA spiked into wild-type DNA (approximately 0.1%) are shown. The mutation assay (FAM channel) was duplexed with the corresponding wild-type reference assay (HEX channel).

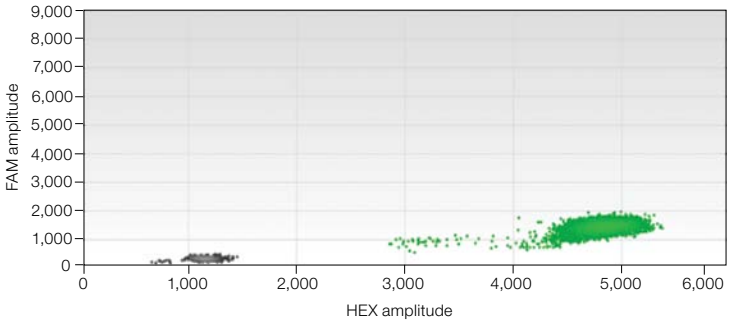

Fig. 2. ddPCR amplitude scatter plot (wild type). Single-well data for wild-type DNA (no mutant) are shown. The mutation assay (FAM channel) was duplexed with the corresponding wild-type reference assay (HEX channel).

Copy Number Assays

An alteration in copy number state with respect to the reference locus is copy number variation. 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 changes, including single nucleotide polymorphism–based microarrays, comparative genomic hybridization, and quantitative PCR, lack the sensitivity and resolution needed for this fine degree of quantitative discrimination in copy number analysis. The massive partitioning of a copy number ddPCR reaction across up to 20,000 droplets enables the fine quantitative discrimination required to resolve small fold changes. This is due to the high precision of the ddPCR concentration measurement and the absolute nature of the measurement.

Assay Specifications

- 62 target assays with a focus on cancer and neurological disorders (Table 2)
- 2 reference assays, which are sold separately and target ultraconserved chromosomal regions (Table 2)
- Universal/single restriction enzyme strategy used for assay design
- Copy number calls within 10% of expected; that is, the ability to determine less than 2-fold changes
- Uniform PCR cycling conditions and primer/probe strategy
- Primer specificity confirmed by next-generation sequencing

Table 2. Target assays for cancer and neurological disorders.

Reference Genes	Target Genes	Target Genes	Target Genes	Target Genes
AP3B1	*APC*	*EGFR*	*MELK*	*RPS6KB1*
EIF2C1	*AR*	*ERBB3*	*MET*	*SHH*
	ARIDIA	*FGFR1*	*MTAP*	*SKP2*
	ATM	*FGFR2*	*MYB*	*SLIT2*
	BIRC2	*FOXO1*	*MYC*	*SMAD4*
	BRCA1	*GAB2*	*MYCN*	*TERT*
	BRCA2	*GRB2*	*NCOA3*	*TSC1*
	CCDN1	*HMGA2*	*NCOR1*	*TSC2*
	CCND2	*IGF1R*	*ORAOV1*	*WHSC1L1*
	CCNE1	*IRS2*	*PARK2*	*WISP*
	CDK4	*JUN*	*PDGFRA*	*YAP1*

Documentation

When a PrimePCR probe assay for ddPCR is ordered online, the following documentation is available:

- Validation data: include gene information, assay design, context sequence following minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines, probe and primer purification information, and sample data
- Instruction manual: includes the assay protocol

For more information, visit us at bio-rad.com/web/PrimePCRddPCRassays.

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