Droplet Digital™ PCR: High-Resolution Copy Number Variation Analysis

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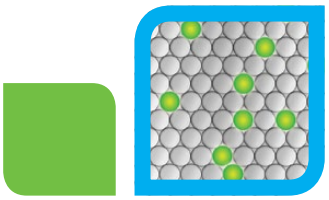

Droplet Digital PCR

Bulletin 6475

Abstract

Copy number variation (CNV), including genomic deletions or duplications, is a prominent source of interindividual variability, and copy number variation of specific loci has been associated with cancers, neurological disease, and adverse drug response. The ability to reliably discriminate between copy number (CN) states across samples and to detect copy number aberrations in heterogeneous samples is crucial for tumor profiling, detecting somatic mosaicism, and population studies. Droplet Digital PCR (ddPCR™) enables high-resolution CNV analysis through ultraprecise, absolute quantification of specific nucleic acid sequences.

Using the QX100™ Droplet Digital™ PCR system, we demonstrate high-resolution CN assessment for multiple targets in samples of homogeneous and heterogeneous genotype. Up to 1.2-fold discrimination between higher-order CN states of the *MRGPRX1* and *CCL3L1* genes in cell lines is shown in Figure 1A. We also assess *CYP2D6* copy number in a heterogeneous sample, demonstrating the ability to discriminate an approximately 5% difference in gene content. The high precision of ddPCR CNV analysis surpasses traditional technologies and offers a low-cost, high-throughput method to validate CN alterations discovered by next-generation sequencing.

Copy Number Variation Analysis by ddPCR in Homogeneous Samples

Methods

DNA HapMap samples (NA11994, NA18507, NA18502, NA19221, NA19205, and NA18916, Coriell Institute) were digested with CviQI (New England Biolabs) at 10 U/µg DNA. *MRGPRX1* and *CCL3L1* target assays (FAM) were duplexed to *RPP30* and *AP3B1* (ultraconserved element) reference assays (HEX), respectively; 33 ng of human genomic DNA was tested per well. *MRGPRX1* and *CCL3L1* data represent three merged technical replicate wells.

Results

Using ddPCR, copy number status of *MRGPRX1* and *CCL3L1*, two gene targets known to vary in CN across individuals, was reliably determined (Figure 1; 95% confidence interval shown). The seven HapMap human genomic DNA samples demonstrate *MRGPRX1* CN evaluation from 1 through 6, and *CCL3L1* up to CN 8. The ability to discriminate the copy number of a CN 6 sample versus a CN 5 sample (*MRGPRX1*) represents a 1.2-fold or 20% difference in DNA content.

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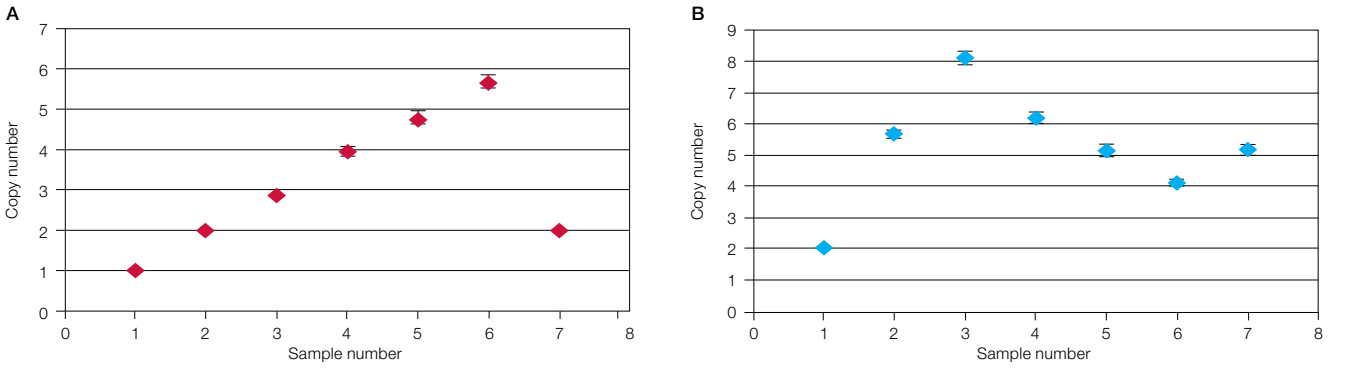

Fig. 1. Determination of copy number for *MRGPRX1* (A) and *CCL3L1* (B) in homogeneous samples using the QX100 ddPCR system.

Copy Number Variation Analysis by ddPCR in Heterogeneous Samples

Methods

DNA HapMap samples (Coriell) with two (NA18916) or three (NA18507) copies of the *CYP2D6* gene, as assessed by ddPCR, were digested with MseI at 10 U/µg DNA. Admixed samples were prepared by titrating high copy number (NA18507, CN 3) DNA into a CN 2 sample (NA18916). A twofold dilution series representing 100%, 50%, 25%, and 12.5% CN 3 DNA in a CN 2 background was tested. A total of 75 ng human genomic DNA was tested per well. *CYP2D6* target assay (FAM; Hs00010001_cn, Life Technologies Corporation) was duplexed to *EIF2C1* (ultraconserved element) reference assay (HEX). Each data point represents four merged technical replicate wells.

Results

With ddPCR, a sample containing 12.5% elevated (CN 3) DNA is easily distinguishable from the 100% wild-type (CN 2) sample (Table 1 and Figure 2). This represents a 6.5% difference in DNA content.

Conclusions

The high precision of ddPCR enables robust CNV assessment using minimal sample under technically challenging circumstances. Resolving copy number differences as small as approximately 5% in heterogeneous samples highlights the power of this technology. Copy number variation ddPCR will empower cutting-edge analyses of cancer genomes and other complex genetic architectures.

Table 1. Copy number measurements of *CYP2D6* in a heterogeneous sample.

Mutant, %	Expected CN	Measured CN	95% CI	Resolution Achieved, %
0	2.00	2.03	(2.00–2.06)	NA
12.5	2.13	2.18	(2.14–2.21)	**6.5**
25	2.25	2.29	(2.25–2.32)	12.5
50	2.50	2.60	(2.56–2.64)	25
100	3.00	3.03	(2.98–3.08)	50

CI, confidence interval; CN, copy number.

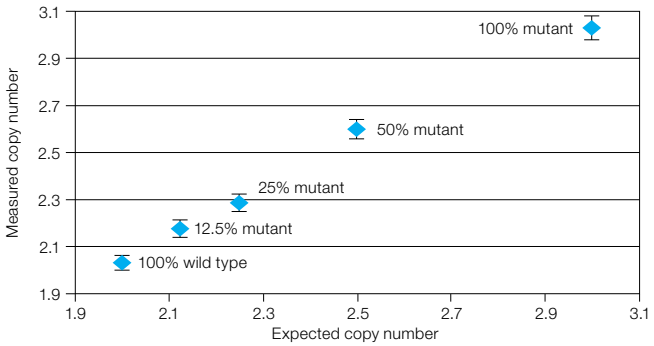

Fig. 2. Determination of copy number for *CYP2D6* in a heterogeneous sample using the QX100 ddPCR system.

For more information, visit
www.bio-rad.com/web/ddPCRcnv.

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