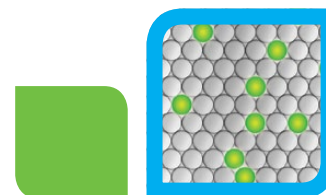


Droplet Digital™ PCR: Ultra-Sensitive Rare Event Detection Using Bio-Rad's QX100™ Droplet Digital PCR System

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Droplet Digital PCR

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Abstract

Droplet Digital PCR (ddPCR™) enables accurate, precise, and sensitive quantification of specific nucleic acid sequences. One of the advantages of ddPCR is that partitioning into droplets allows effective increase of rare target abundance within an individual compartment, and thus facilitates rare event detection (RED), which includes two classes:

- Rare mutation detection (RMD)
- Rare sequence detection (RSD)

Using the QX100 ddPCR system, we show that *BRAF V600E* rare mutant allele can be detected in 200,000 times wild-type background (0.0005% mutant) with a duplex TaqMan assay. For RSD, we demonstrate that one human herpesvirus 6 (HHV-6) can be readily detected among 1,000,000 genome copies of human DNA (0.0001% rare sequence) with a single TaqMan assay. The sensitivity of the QX100 system for RED makes it an ideal tool for screening cancer cells with rare somatic mutations, and for detecting foreign species in a biological sample.

Rare Mutation Detection: Detection of 0.0005% *BRAF V600E* Mutant DNA

Experiment Setup

Set up two 96-well plates with:

- 8 wells of positive control using mixed wild-type and mutant DNA at known concentrations
- 16 wells of no template control
- 120 wells of negative control using wild-type DNA at 5,000 copies/μl
- 48 wells of RMD test wells using wild-type DNA at 5,000 copies/μl with mutant DNA at 0.025 copies/μl spiked in

Results

Two false-positive droplets in 120 negative control wells and 15 positive droplets in 48 RMD test wells were observed. The number of positive and negative droplets and calculated concentrations for wild-type and mutant DNA in 48 RMD wells are shown in Table 1.

Table 1. Results from 48 RMD test wells.

DNA	Positive Droplets	Negative Droplets	Tested Concentration (95% CI), copies/μl
Wild-type DNA	565,907	6,503	4,920 (4,890–4,950)
Mutant DNA	15	572,393	0.029 (0.017–0.046)
Ratio (mutant DNA/wild-type DNA × 100%)			0.00059%

CI, confidence interval.

Rare Sequence Detection: Detection of 0.0001% Viral DNA in Human Genomic DNA

Method

At least 3,000,000 human genome equivalents (GEs) were screened to ensure detection of one viral DNA/million human GEs. A singleplex assay was used to detect viral DNA in a

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high-concentration background of human DNA while the human DNA was quantified separately at a 25-fold dilution with an *RPP30* assay (Figure 1).

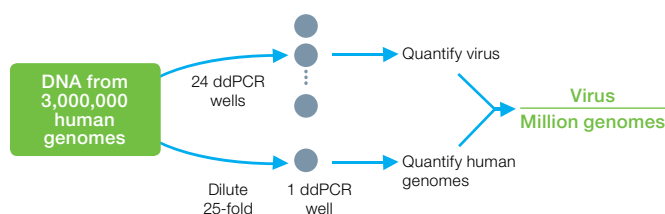


Fig. 1. Screening method for detection of viral DNA in human genomic DNA.

Experiment Setup

Set up one 96-well plate with:

- 4 wells of positive control using mixed viral and human genomic DNA at known concentrations
- 8 wells of no template control
- 60 wells of negative control using human genomic DNA at 25,000 copies/μl
- 24 wells of RSD test wells using human genomic DNA at 25,000 copies/μl with HHV-6 viral DNA at 0.025 copies/μl spiked in

Results

Zero false-positive droplets in 60 negative control wells and four positive droplets in 24 RSD test wells were observed. The number of positive and negative droplets and calculated concentrations for HHV-6 and human genomic DNA in 24 RSD test wells are shown in Table 2.

Table 2. Results from 24 RSD test wells.

DNA	Positive Droplets	Negative Droplets	Tested Concentration (95% CI), copies/μl
HHV-6 DNA	4	276,817	0.0159 (0.00479–0.0374)
Human genomic DNA (25-fold dilution)	7,730	4,469	1,100 (1,080–1,130)
Human genomic DNA (calculated)			27,500 (27,000–28,250)
Ratio (HHV-6 DNA/human DNA x 100%)			0.000058%

CI, confidence interval.

Discussion: False-Positive Rate

In these experiments, positive samples were identified on the basis of a small number of positive droplets. When following this process, it is important to run a sufficient number of negative controls to ensure that there is a statistically significant separation between the negative controls and positive samples. False-positive droplets in negative controls have multiple root causes: contamination, assay design, and instrumentation. In our experience, contamination prior to droplet formation is the most significant contributor to false-positive droplets because of the high specificity and sensitivity of the assay. After droplet formation, contamination is not an issue.

Conclusions

Bio-Rad's QX100 ddPCR system offers extra sensitivity for rare event detection. As low as 0.0005% rare mutant and 0.00013% rare sequence were reliably detected.

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

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