



Droplet Digital™ PCR Success Story

Genomic Variation Analysis: Copy Number Variation

DUF1220 Copy Number Variation across Healthy and Diseased Populations

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“Our laboratory is interested in the development and application of advanced genome technologies, particularly as they apply to our understanding of human evolution and human disease.”

Research Background

Areas of special interest in our laboratory include the identification of genetic factors involved in neurogenetic diseases such as microcephaly, macrocephaly, autism, schizophrenia, and other disorders of cognitive function. According to our research, *DUF1220* plays a role in human brain size evolution as well as in brain size pathologies. *DUF1220* is a highly duplicated sequence that can promote misalignment during meiosis, for example, which can cause duplications or deletions of *DUF1220* sequences as well as flanking genes.

Technologies that are used include array comparative genomic hybridization (array CGH), next-generation sequencing of DNA and RNA, Droplet Digital PCR (ddPCR™), gene expression profiling, and novel bioinformatics tools for the rapid in silico discovery of genes underlying quantitative trait loci for complex traits.

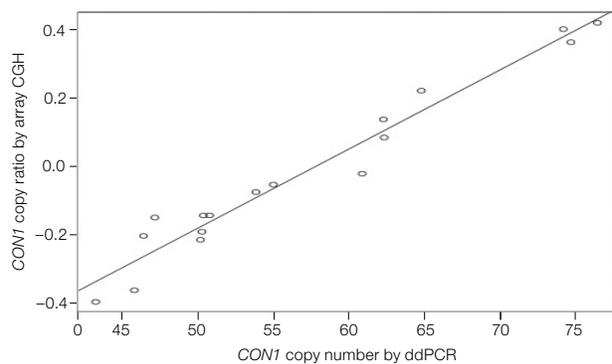
Application

Our primary application for the QX100™ Droplet Digital™ PCR system is to determine copy number variation (CNV) within a particular highly duplicated region of the genome. One of our assays is designed to detect approximately 200 diploid copies of our amplicon of interest in control samples and a variation of the number in test samples. We needed a method to validate array CGH data and test many additional samples. Array CGH is cost prohibitive when it is used to test hundreds of samples. Originally, we had developed qPCR assays that provided acceptable results to validate large duplications or deletions, but those assays lacked the measurement precision and accuracy to detect small differences between individuals. We hoped the QX100 ddPCR system could help us assay those small copy number differences between individuals with higher precision.

ddPCR Results

We have been able to use our TaqMan qPCR assays to obtain very reproducible results on the QX100 ddPCR system with several CNV assays, including our highest CNV assay of approximately 200 diploid copies/genome. High CNV assays such as this require significant fine tuning of assay variables, particularly DNA concentration, in order for the test and reference duplex reaction assays to both fall within the ideal range of the instrument. The methods and instrument are robust, leaving little variation in results from user to user once trained. We are continuing to optimize several aspects of our assays and believe our results will continue to improve, though they are currently much improved compared to qPCR assays.

Correlations of CNV determined by ddPCR, with a copy ratio determined by array CGH, are consistently approximately 0.90 and as high as 0.98. We believe that with slight improvements in our assays, correlations of 0.95 should be consistently observed. Additionally, reproducibility, as measured by intraclass correlation coefficient, has been consistently high, ranging from 0.75 to 0.95 (0.75 is considered excellent).



Correlation of array CGH and ddPCR methods. Array CGH, array comparative genomic hybridization; ddPCR, Droplet Digital PCR.



Conclusions

Bio-Rad's QX100 ddPCR system provides a method for accurately quantifying copy number, providing results that our laboratory has been hoping to find for a long time. ddPCR has made it possible to ask CNV questions requiring increased precision and across a greater variety and number of samples than we had previously been able to. For difficult questions such as ours, this technology is extremely valuable.

“To date, ddPCR has successfully allowed us to quantify the highly duplicated *DUF1220* sequences within about a 10% error range.”

Publications

Dumas LJ et al. (2012). DUF1220-domain copy number implicated in human brain-size pathology and evolution. *Am J Hum Genet* 91, 444–454.

O'Bleness MS et al. (2012). Evolutionary history and genome organization of DUF1220 protein domains. *G3* 2, 977–986.

Popesco MC et al. (2006). Human lineage-specific amplification, selection, and neuronal expression of DUF1220 domains. *Science* 313, 1304–1307.

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Practice of the polymerase chain reaction (PCR) may require a license.

For more information, visit

www.bio-rad.com/ddPCRsuccessAnderson.

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