Droplet Digital™ PCR Success Story

Genomic Variation Analysis: Single Nucleotide Polymorphism

Validation of Allele-Specific Expression Predicted by RNA-Seq in Human Brain Specimens

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Research Background

The goal of our research is to study genetic mechanisms contributing to the specialization of the human brain in the context of human development and disease occurrence. Technologies used in our laboratory include high-throughput sequencing, microarray, Droplet Digital PCR (ddPCR™), various bioinformatics tools, and gene manipulation in model organisms to functionally validate these findings. Allele-specific expression (ASE) is an important factor contributing to human phenotypic variability and the development of complex traits and diseases. We have employed RNA-Seq and SNP chip technology to predict ASE of 16 different brain regions from six healthy adult individuals.

Application

Among candidate alleles with an imbalanced expression from the six healthy individuals, one allele failed to be validated for its genotype by a standard quantitative PCR (qPCR) approach using an inventoried TaqMan assay. The homozygous A/A allele was not successfully resolved due to amplification of the G allele (likely occurring from nonspecific, cross-reactive amplification), generating ambiguous results (Figure 1). Several attempts with different settings yielded the same results. When I was going to move to some other technologies to overcome this problem, the QX100™ Droplet Digital PCR system was launched in our laboratory. We primarily use the ddPCR system to quantify absolute gene expression levels, especially for genes with low expression. I hoped the QX100 ddPCR system could also help assay genotype identification and allelic imbalance with higher precision.

ddPCR Results

As I expected, the QX100 ddPCR system generated a crystal clear, unambiguous result for genotype identification (Figure 2). Results show that the homozygous A/A allele generated nearly zero positive droplets using a G assay (FAM) while generating twice as many droplets with an A assay (VIC) when the data were normalized by their input genomic content (Figure 2). I also saved our precious human genomic DNA (gDNA) by applying ten times less gDNA than the amount used for qPCR. The methods and instrument are robust, producing little variation in results among users once they are trained. I have applied ddPCR to quantify ASE events in cDNA samples obtained from various brain regions of different individuals and I achieved an absolute comparison of gene expression from both alleles (data not shown) without using any standard.

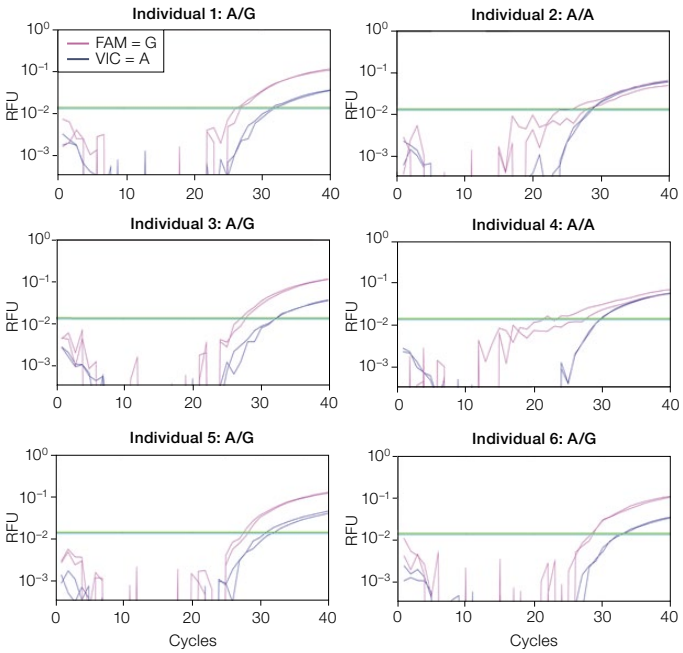

Fig. 1. Real-time PCR amplification curves for a candidate ASE allele. gDNA SNP assays were performed using qPCR. qPCR failed to call the A/A genotype. RFU, relative fluorescence units.

* Work was completed at Nenad Sestan Laboratory, Department of Neurobiology, Yale University School of Medicine.

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Sample	Description	Sample	Description
DO3	Individual 1 (A/G)	DO6	Individual 4 (A/A)
DO4	Individual 2 (A/A)	DO7	Individual 5 (A/G)
DO5	Individual 3 (A/G)	DO8	Individual 6 (A/G)

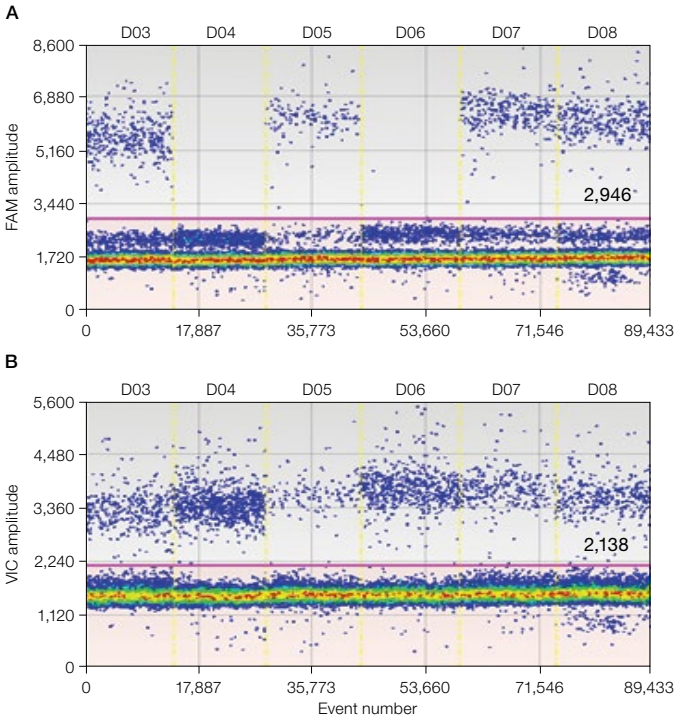

Fig. 2. Droplet Digital PCR validates both A/G and A/A genotypes. gDNA SNP assays were performed with G (**A**, FAM) and A (**B**, VIC) assays using ddPCR. A/G and A/A genotypes were successfully validated by ddPCR.

Conclusions

Bio-Rad's QX100 ddPCR system provides a method for accurately validating genotypes (SNPs). It also allows direct quantification of ASE (allelic imbalance) without the use of a standard, as opposed to standard qPCR that can provide indirect quantification. The costs and time constraints of ddPCR are negligible compared to other known methods, such as the SNaPshot multiplex system, for accurate ASE detection.

"Droplet Digital PCR outperforms qPCR in accurate genotype validation. It can also be used to quantify allele-specific expression events."

Publication

Kang HJ et al. (2011). Spatio-temporal transcriptome of the human brain. Nature 478, 483–489.

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

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