Droplet Digital™ PCR Success Story

Gene Expression

Digital Quantification of Potential Therapeutic Target RNAs

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"Being able to accurately establish the number of RNA molecules per cell provides a foundation for understanding how RNA functions."

Research Background

My laboratory is interested in using antisense oligonucleotides and duplex RNAs to control gene expression. We have observed that duplex RNAs can target sequences within gene promoters and either repress or activate gene expression in cultured human cancer cells. These studies suggest that double-stranded RNA can recognize sequences within chromosomal DNA and that RNA-mediated control of gene expression is even richer than had been previously imagined. Our projects include allele-selective targeting of trinucleotide repeat disease genes, such as huntingtin and ataxin-3, and gene activation through recognition of nuclear noncoding RNAs.

Application

Accurate determination of the amount of a given RNA within a cell is necessary to gain a full understanding of the RNA's function and regulation. Typically, the abundance of RNA is measured by quantitative PCR (qPCR). With qPCR, however, absolute quantification is not possible unless an adequate reference standard curve is generated. The method is not well suited for detecting low copy number templates and values vary depending on the specific primers used. To overcome these drawbacks digital PCR has been developed to obtain exact values for RNA copies in a sample. We use Droplet Digital PCR (ddPCR™) to quantify long noncoding RNAs (lncRNAs) from various subcellular compartments within human cells. Accurate quantification of lncRNA abundance may suggest function and discriminate between long noncoding RNAs that have a biological role and those that may be biological noise due to the minute action of RNA polymerase.

ddPCR Results

Droplet Digital PCR aims to provide a more direct measurement of RNA copy number. We found the dynamic range of ddPCR to be approximately 10–10,000 copies/µl reaction mixture. This corresponds to threshold cycle (C_T) values of approximately 18–28. Therefore, we find that results obtained using ddPCR parallel those from qPCR (Figure 1).

A clear role for lncRNA controlling *COX2* expression has been demonstrated and because of the therapeutic relevance of the gene with regard to inflammatory response we used ddPCR to characterize its expression levels. We were interested in the amount of lncRNA throughout the cell, and therefore obtained extracts from cytoplasm, nuclei, and further purified nuclei to obtain nucleoplasmic (soluble nuclear) RNA and RNA associated with chromatin. All these samples were analyzed by both ddPCR and qPCR.

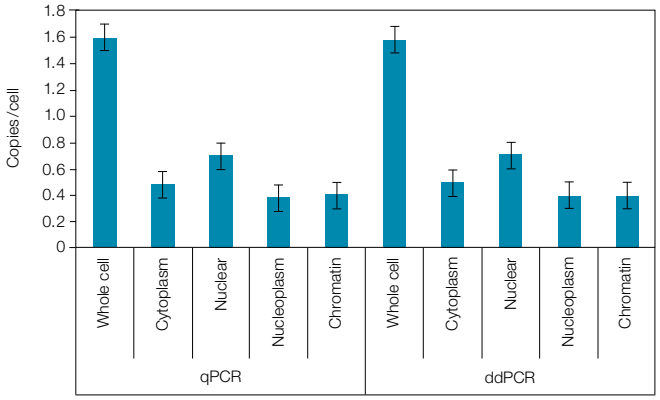

Fig. 1. Quantification of cyclooxygenase 2 long noncoding RNA by qPCR and ddPCR. There is good agreement in total copy number per cell and distribution across compartments. Experiments were performed in triplicate. Standard deviations are shown. ddPCR, Droplet Digital PCR; qPCR, quantitative PCR.

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Conclusions

Relatively few researchers measure absolute values for RNA molecules per cell. Such numbers, however, can have a big impact on the function of the RNA. For example, an RNA that is present in just one or two copies per cell is unlikely to function in trans relative to its target. Such rare RNAs are more likely to function in cis, at the gene that encodes them. By contrast an RNA that is present in hundreds or thousands of copies per cell is a better candidate for action in trans. ddPCR allows accurate determination of these values and it can be used to help build a firm foundation for understanding disease mechanisms.

"Our results show that ddPCR can be a robust tool for identifying the number of RNA species inside cells."

Publication

Dodd DW et al. (2013). Digital quantitation of potential therapeutic target RNAs. Nucleic Acid Ther 23, 188–194.

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