Droplet Digital PCR enables reproducible quantification of microRNA biomarkers

A study published online in *Nature Methods* today demonstrated that Droplet Digital PCR (ddPCR) technology can be used to precisely and reproducibly quantify microRNA (miRNA) in plasma and serum across different days, paving the way for further development of miRNA and other nucleic acids as circulating biomarkers.

"In the field of circulating microRNA diagnostics, droplet digital PCR enables us to finally perform biomarker studies in which the measurements are directly comparable across days within a laboratory and even among different laboratories," said Dr. Muneesh Tewari, Associate Member in the Human Biology Division at the Fred Hutchinson Cancer Research Center and lead author of the study.

Challenges in miRNA quantification

miRNAs are small regulatory RNA molecules with diverse cellular functions. The human genome may encode over 1,000 miRNAs, which could target about 60 percent of mammalian genes. Because they are abundant in many cell types, exist in highly stable extracellular forms, and may provide direct information about disease processes, they are being actively studied as blood-based biomarkers for cancer and other diseases.

Quantitative real-time PCR (qPCR) has been used for the analytical measurement of miRNAs in blood samples; however, researchers have found that qPCR measurements of miRNAs in serum or plasma display unacceptably high interday variability, undermining the use of miRNAs as reliable blood-based biomarkers. An approach that yields more dependable results has therefore been sought by researchers in this field.

Advantages of ddPCR for miRNA detection

Digital PCR has many advantages over qPCR including the ability to provide absolute quantification without a standard curve and robustness to variations in PCR efficiency across different samples and assays. These and other advantages are embodied in Bio-Rad Laboratories' QX100™ Droplet Digital PCR (ddPCR) system, which was introduced in 2011.

"We chose to use Bio-Rad's QX100 Droplet Digital PCR system because it was the first system on the market that could make digital PCR practical from a cost and throughput standpoint for routine use in the lab," said Dr. Tewari.

To assess the imprecision introduced by each workflow step—serial dilution preparation, reverse transcription (RT), and PCR technical replicates—Dr. Tewari and his team conducted nested analyses of ddPCR vs. qPCR on cDNA from a dilution series of six different synthetic miRNAs in both water and plasma on three separate days. In comparison to qPCR, the researchers found that ddPCR demonstrated greater precision (48–72% lower coefficients of variation) with respect to PCR-specific variation

Next, the team performed a side-by-side comparison of qPCR to ddPCR for detecting miRNAs in serum. They collected sera samples from 20 patients with advanced prostate cancer and 20 age-matched male controls and measured the abundance of miR-141, which has been shown to be a biomarker for advanced prostate cancer. Samples were analyzed by qPCR and ddPCR with individual dilution series replicates prepared on three different days. They found that ddPCR improved day-to-day reproducibility seven-fold relative to qPCR. It was also able to demonstrate differences between case vs. control specimens with much higher confidence than qPCR ($p=0.0036$ vs. $p=0.1199$).

"Droplet digital PCR will allow us to accurately follow serum microRNA biomarker concentrations over time during a patient's treatment course, something that has been nearly impossible to achieve with real-time PCR," he said.

More information: Paper: dx.doi.org/10.1038/nmeth.2633
Droplet Digital PCR (ddPCR): www.bio-rad.com/en-us/category/digital-pcr-technology

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