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Amplifying DNA, One Droplet at a Time

Droplet digital PCR is helping to advance complex products—from gene therapies to customized cancer drugs.

It was nearly 40 years ago that biochemist and Nobel Laureate Kary Mullis developed the DNA replication technique known as polymerase chain reaction (PCR), now the most widely used method in molecular biology.

One cannot emphasize enough how PCR has changed the dynamics of lab work, particularly in the areas of forensics, cancer, genetic disorders, and infectious diseases. PCR enables us to amplify the errant sequences associated with the diseases and without it we would not be able to develop the gene therapies that have taken the field of large molecule drugs by storm.

Before PCR came along, the only way to produce multiple copies of a gene was to clone the DNA—a time-consuming process involving inserting a gene of interest into living bacterial cells and replicating the gene along with the DNA during the division and replication process.

PCR changed that dynamic but the Wow Factor hasn't stopped there. Researchers continue to find new and innovative applications for PCR, and new iterations of the technology. The most powerful approach is droplet digital PCR (the subject of this blog).

Droplet digital PCR (ddPCR) is a form of digital PCR that works like conventional PCR except that it partitions DNA samples into thousands of separate reaction chambers. The "droplet" comes from the method used—a water-oil emulsion that divides samples into 20,000 droplets. PCR amplification occurs in every droplet and the compartments that end up containing the target molecule complete the PCR and read "positive" while the ones containing no DNA register as negative. This is where the term digital comes in, as it is a yes or no answer, or 0-1.

Unlike chromatography or ligand binding assays where the intensity of a signal is measured in parallel with calibration curves, ddPCR gives the means to count the absolute number of DNA or RNA molecules in each sample, theoretically making the test more precise and sensitive.

Assessing the viral vectors in a gene therapy

Gene therapies—which involve the transfer of genetic material, usually a carrier or vector, and the introduction of the gene into the appropriate cells—is one of the biggest areas for ddPCR, though many labs still use the conventional PCR method.

One key area for ddPCR is in assessing the biodistribution of the viral vectors used to chauffeur gene therapies into cells. When treating an animal or human with a viral vector-based gene therapy, it's important to quantify the amount of vector remaining in the tissue because if the concentration of virus is too high it might cause harm, and if it is too little the gene therapy might not work.

Standard PCR tackles this question by obtaining a correlation between the signal and the concentration of the substance, and then trying to link them. Digital PCR avoids this challenge because a calibration curve is not needed to base the results on. With ddPCR, DNA or RNA molecules in the sample are literally counted, for an absolute and very sensitive quantitative result.

Measuring Mutated DNA

Of course, ddPCR is not just useful in assessing gene therapies' genome copy numbers. It is also uniquely valuable in determining mutations in certain tumor cells or patient blood. Typically, labs have looked for tumor cell DNA in plasma. The problem with this approach is that the levels of mutated DNA are so low that they are difficult to distinguish from normal DNA in plasma. In other words, if conventional PCR is used to detect mutated DNA, the signal will be lost among the noise emanating from normal cells DNA.

With ddPCR, this challenge can be circumvented by isolating the mutated and unmutated DNA in different droplets, making the results easier to detect.

Cell Therapies

When it comes to cell therapies, ddPCR also has value. Some cell therapies involve a sort of directed mutation where a pool of cells is taken from a patient and modified to give them therapeutic properties. These cell therapies can be then readministered to the same patient. What is interesting about ddPCR is that the cell therapy can be better characterized because the percentages of cells that have been modified can be easily determined.

What do regulatory authorities say about ddPCR?

Science often moves faster than regulations, and PCR applications are no exception. While regulatory authorities do recognize that PCR is the most adequate technique for assessing biodistribution for gene and cell therapies, they do not distinguish which type of PCR is best and there are no specific requirements that specify which method to use. What is important is harmonization of systems to ensure consistent results.

In Canada, we were the first site to offer ddPCR, and six of our sites in the US and Europe also offer it. All the procedures are harmonized—from instrument qualification to systems validation, meaning that an assay can be transferred easily from one site to another and expect the same results. We can also transfer assays from clients or design assays from scratch. This could go a long way toward ensuring that these advanced products move forward with minimal disruption.

Conclusion

Gene and cell therapies have come of age, with several products approved and numerous products in development. These therapies require specific laboratory investigations, among which ddPCR is a tremendous asset to obtain reliable analytical results – necessary along the long way to product approval.

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