Droplet Digital™ PCR Success Story

Viral Quantification

Adeno-Associated Virus Vector Genome Titer Assay

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"Our laboratory studies adeno-associated virus vectors for human gene therapy."

Research Background

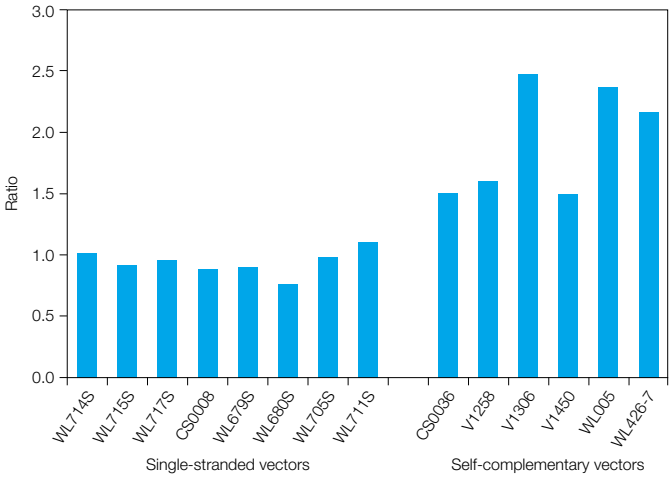
Recombinant adeno-associated virus (AAV) is rapidly becoming the vector of choice for human gene therapy applications due to the ability to achieve prolonged gene expression in a variety of tissues without a significant immune response or toxicity. Our laboratory has discovered a number of novel AAV serotypes with enhanced gene expression and alternate tissue tropisms and has pioneered the study of these vectors for gene delivery. Some of these novel serotypes have been tested as gene delivery vehicles in clinical trials with great success and the first AAV-based gene therapy product was recently approved in Europe for use in humans.

Application

Large-scale manufacture of AAV vector for clinical trials requires a high degree of sophistication in both process design and lot release assays. Perhaps the most critical lot release assay for AAV vector preparations is the AAV vector genome (vg) titer assay, since genome copy numbers are universally used for dosing purposes in both preclinical and clinical studies. The most prevalent method for quantifying AAV vectors is currently quantitative PCR (qPCR). However, the reliance of this method on a standard curve and the potential impairment of DNA amplification efficiency from poor primer design, the presence of inhibitors, and template secondary structure can lead to variance and under-reporting of genome copy numbers. Absolute determination of vg titers by Droplet Digital PCR (ddPCR™) eliminates the need for a standard curve and may offer advantages over qPCR since low-efficiency PCR reactions are still scored as positive. In addition, the unparalleled degree of precision demonstrated by ddPCR in a variety of DNA applications would seem to make the technique a highly suitable candidate for the titration of clinical-grade AAV vector preparations.

ddPCR Results

We compared an AAV genome titer assay based on ddPCR with a standard and an optimized qPCR assay for the titration of both single-stranded (ss) and self-complementary (sc) AAV genomes. We demonstrated absolute quantification of ssAAV vector genomes by ddPCR with up to fourfold increased sensitivity over a standard qPCR titration but with equivalent sensitivity to an optimized qPCR assay. In the case of AAV vectors with self-complementary (double-stranded) genomes containing a high degree of secondary structure, ddPCR titers were on average fivefold, 1.9-fold, and 2.3-fold greater than those determined by standard qPCR, optimized qPCR, and agarose gel assays, respectively. Genome titering based on ddPCR was significantly superior to qPCR in terms of both intra-assay coefficient of variation (CV of 2%) and inter-assay precision (CV of 5%). In addition, we were able to demonstrate that ddPCR is at least tenfold more resistant to the presence of PCR reaction inhibitors than qPCR.



Ratios of ddPCR vg titers obtained for different AAV vector lots versus optimized qPCR.

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Conclusions

We find that ddPCR represents a highly precise method to determine AAV vector genome titers and is not unduly influenced by inefficient amplification or standard curve issues. The technique delivers a universal assay for all AAV vector types without some of the limitations of qPCR or the need to resort to separate methods. While the technique does not eliminate the PCR inhibition seen with primer-probe sets targeted close to the terminal hairpin of scAAV vectors, the moderate increase in sensitivity observed in our best qPCR assay and increased intra- and interassay precision using ddPCR make the ddPCR assay highly suitable for genome copy titration of these vectors. This is likely especially important in clinical or commercial vector production where the highest degree of reproducibility and precision is required and where small titer differences can translate to fewer scaled production runs and significant financial savings. The high degree of precision of the ddPCR assay also allows running fewer replicates and thus reduces the number of analyses of multiple samples and primer-probe sets on a single 96-well plate. The lack of requirement for a plasmid standard curve eliminates the time, expense, and error involved in its preparation.

> "ddPCR confers some major advantages for vector genome quantification, which will be important for the production and use of clinical AAV gene therapy vectors."

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

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