Transitioning Your Assay from Quantitative PCR to Droplet Digital PCR

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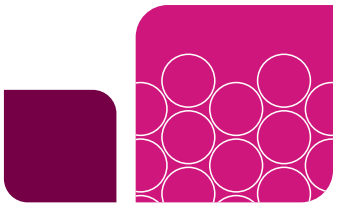

Droplet Digital PCR

Bulletin 7320

Abstract

This application note will discuss migrating assays from real-time quantitative PCR (qPCR) to Droplet Digital PCR (ddPCR). These two PCR methods determine the concentration of sample DNA using fundamentally different approaches and a bridging study between the two methods presents data demonstrating the differences and similarities to consider. In qPCR, concentration measurements from the bulk PCR reaction are relative to a control or standard DNA material because the fluorescent signal is based on the proportionality between fluorescence and DNA amount. In Droplet Digital PCR, DNA concentration is determined directly by partitioning the PCR reaction into thousands of individual droplets that are counted as positive or negative and evaluated with Poisson statistics after PCR amplification is complete. Here we show data for an adeno-associated virus (AAV) inverted terminal repeat (ITR) assay to provide guidance for migrating a qPCR assay that uses a standard curve to a ddPCR assay that directly quantifies a sequence without a standard curve. Initially, a temperature gradient during the annealing/extension step of a ddPCR reaction was used to maximize the separation between positive and negative droplets. Subsequently, a restriction enzyme was used to illustrate the effect of amplicon accessibility or linkage on the concentration. Ultimately, a combination of process-related and experimental factors can contribute to the differences in viral titers between qPCR and ddPCR platforms due to assumptions inherent in each technique. Transitioning from quantitative PCR to Droplet Digital PCR can be simple and provide consistent, robust ddPCR results that can be directly compared to past qPCR data by conducting a straightforward bridging study.

Introduction

PCR is a powerful technique that can be used to detect, compare, and quantify a specific sequence of DNA or RNA by exponentially amplifying the starting amount millions of times. The basic steps of PCR are DNA denaturation, annealing of DNA primers designed specifically for the DNA sequence of interest, and primer extension by Taq polymerase. This cycle of denaturation, annealing, and extension is repeated 35–40 times until the PCR reaction is complete. The exponential amplification is necessary to magnify the DNA to a level that is detectable.

Since the invention of PCR, many different methods have been developed to detect amplified DNA. Here we will discuss three common methods: gel-based detection, qPCR detection, and ddPCR detection. These three methods have advanced in their sensitivity and ability to quantify the starting DNA.

The earliest detection and quantification methods of PCR reactions were based on agarose gel electrophoresis and DNA staining after the PCR reaction was complete. Visual comparisons of bandwidth and brightness would be used to quantify amounts of DNA in a sample by referencing a DNA control sample on the same gel. This relative method using a standard or control to quantify DNA is still used today, despite the large error in the measurement.

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Real-time quantitative PCR measures the fluorescent signal after each cycle during PCR amplification. After the PCR reaction is complete, data from samples of interest are compared with data from a reference sample to determine the amount of DNA in the samples of interest. This method can detect and quantify DNA with a much lower error than gel electrophoresis. As in gel electrophoresis, qPCR measurements are relative to the control or standard DNA material because the fluorescent signal is based on the proportionality between fluorescence and DNA amount. This method is dependent on the identity and quality of the standard curve material and susceptible to operator error. In many applications it is difficult to create a standard curve material that mimics the sample well enough to produce accurate quantities. Additionally, batch-to-batch differences of standard curve materials must be qualified and accounted for.

Droplet Digital PCR partitions each PCR reaction into thousands of individual droplets. Each droplet is an individual PCR reaction that is counted as positive or negative after the PCR amplification. This method physically counts the DNA instead of comparing it to another DNA sample and therefore does not require a standard curve to assign the quantity.

There are similarities and differences between quantitative PCR and Droplet Digital PCR. Here we will show data that provide guidance for migrating a qPCR assay that uses a standard curve to a ddPCR assay that directly quantifies a sequence without a standard curve.

Materials and Methods

qPCR Data

The AAVpro Titration Kit for Real Time PCR (Takara Bio Inc., 6233) was used to determine the titer of a control AAV2-CMV-GFP viral sample (Vigene Biosciences, CV10004). The protocol initially incubates the virus with DNase I (New England Biolabs, Inc., M0303) for 30 min at 37°C prior to extracting the vector genome using a lysis buffer at 70°C for 10 min. This sample was separated into two aliquots and used for parallel analysis by quantitative PCR and Droplet Digital PCR. For all qPCR reactions, the protocol's recommendations, as summarized below, were followed. After capsid lysis, tenfold serial dilutions of the vector genome in the provided dilution buffer were used directly as the template in qPCR. Samples for a standard curve were prepared from the provided positive control using a tenfold serial dilution with the dilution buffer. According to the provided information, the final copies per reaction of the standard samples ranged from 10^7 to 10^3. The positive control was stated by the vendor to be adjusted to 2×10^7 copies/µl based on an absorbance value at 260 nm (A_{260}), and was described as a plasmid DNA that includes the same ITR copy number as the AAV vector genome. Restriction enzyme–digested samples had 5 U of MspI (New England Biolabs, R0106) per reaction. All samples were assayed in triplicate using a CFX96 Touch Deep Well Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., catalog #1854095) with the recommended two-step thermal cycling protocol that consisted of an initial denaturation at 95°C for 2 min followed by 35 cycles of 95°C for 5 sec and 60°C for 30 sec. A melt curve step (65–95°C incremented by 0.5°C every 5 sec) was added after the amplification. A standard curve was constructed using the quantification cycle (Cq) values and the logarithm of the starting quantity from the positive control samples. The slope and y-intercept of the standard curve regression line were used to calculate the copy number of the control AAV2-CMV-GFP viral sample. Data were analyzed using CFX Maestro Software (Bio-Rad, #12004110).

ddPCR Data

The positive control and control AAV2-CMV-GFP viral sample preparation followed the Takara protocol for the DNase I and lysis steps (described in qPCR data methods section above) and were then serially diluted tenfold in polyA buffer (10 mM Tris-HCl, pH 8, 0.1 mM EDTA, 100 µg/ml polyA) instead of the provided Takara dilution buffer and used directly as the template in Droplet Digital PCR. The primers from the AAVpro Titration Kit were used in ddPCR reactions with QX200 ddPCR EvaGreen® Supermix (Bio-Rad, #1864033). Samples prepared with the Takara protocol were tested with Droplet Digital PCR using primers from the AAVpro Titration Kit, a C1000 Touch Thermal Cycler (Bio-Rad, #1851197), and the QX200 ddPCR EvaGreen® Supermix Product Insert (Bio-Rad, 10028376) with an annealing/extension step that flanked the recommended 60°C temperature gradient from 65 to 55°C. A second control AAV2-CMV-GFP viral sample was prepared using an in-house Bio-Rad protocol. This protocol digests unencapsidated DNA with DNase I for 30 min at 37°C in DNase I digestion buffer containing 0.1% Pluronic F-68 Non-Ionic Surfactant (Thermo Fisher Scientific Inc., 24040032) before serial tenfold dilutions in Eppendorf DNA LoBind Tubes (Sigma-Aldrich, Inc., 0030108051) with polyA buffer. Restriction enzyme–digested samples had 5 U MspI per reaction. Droplets were generated using a QX200 Droplet Generator (Bio-Rad, #1864002) with DG8 Cartridges (Bio-Rad, #1864007), transferred to a 96-well plate, covered with pierceable foil, and heat-sealed with a PX1 PCR Plate Sealer (Bio-Rad, #1814000). The Bio-Rad protocol and ITR assay were run with ddPCR Supermix for Probes (No dUTP) (Bio-Rad, #1863023) and used for the final titer comparisons. The Bio-Rad protocol is a probe-based assay that uses AAV-ITR2 (Bio-Rad, Assay ID dEXD12170542). Droplets were read with the QX200 Droplet Reader (Bio-Rad, #1864003) and the data were analyzed with QuantaSoft Software, version 1.7 (Bio-Rad, #1864011).

Absorbance Measurements

Plasmid, pcDNA3.1(+) Mammalian Expression Vector (Thermo Fisher Scientific, V79020) was measured directly as provided or after being purified with a Monarch PCR & DNA Cleanup Kit (New England Biolabs, T1030). The absorbance of the plasmid before and after column cleanup was measured at 260 nm in triplicate using a Nanodrop 2000c Spectrophotometer (Thermo Fisher Scientific, ND-2000C). The A_{260} values were converted to concentrations (ng/µl) with either a generally accepted multiplicative factor of 50 or an experimentally estimated multiplicative factor of 46.25 (Nwokeoji et al. 2017):

$$\text{Concentration (ng/µl)} = A_{260} \times 50$$

or

$$\text{Concentration (ng/µl)} = A_{260} \times 46.25$$

The number of plasmid copies was calculated by converting nanograms to copy number using the formula:

$$\text{Copies per µl} = \frac{(\text{concentration [ng/µl]}) \times (6.022 \times 10^{23} \text{ molecules/mol})}{(\text{number of base pairs} \times 660 \text{ g/mol}) \times (1 \times 10^{9} \text{ ng/g})}$$

As an orthogonal method to measure n ucleic acid concentration, the plasmid concentration was determined with Droplet Digital PCR using assays targeting the CMV enhancer and SV40 polyA signal sequence. Samples were prepared in ddPCR Supermix for Probes (No dUTP) containing 5 U HaeIII (New England Biolabs, R0108) per reaction and PCR-amplified using the recommended thermal cycling protocol (Bio-Rad, 10026868). The CMV enhancer and SV40 polyA concentrations were averaged and the change of the ddPCR concentration relative to the estimated concentration based on the absorbance at 260 nm was calculated using the formula:

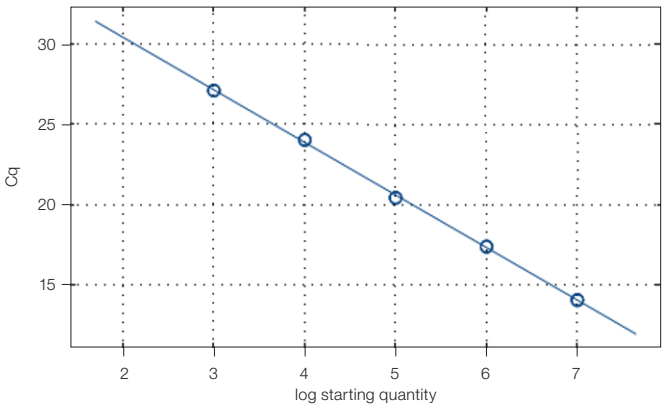
$$\text{Relative change} = \frac{(\text{copies/µl from ddPCR}) - (\text{copies/µl from } A_{260})}{(\text{copies/µl from } A_{260})}$$

Results

Figure 1 shows qPCR data for a standard curve and an AAV2-CMV-GFP viral sample using a commercially available kit and protocol. Each serial tenfold dilution of the standard curve (Figure 1A) from 10^7 to 10^3 copies per reaction was assayed in triplicate as recommended. The parameters from the linear fit of the data and the calculated efficiency are indicated on the graph. The R^2 value (0.999) and the efficiency (102.2%) are within the range for an optimized qPCR experiment. The amplification plot for an AAV2-CMV-GFP viral sample (Figure 1B) shows data in triplicate for a serial tenfold dilution of the virus. The four viral dilutions, expressed in viral genomes, that were within the standard curve concentrations were used to calculate a viral titer of $(3.98 \pm 0.77) \times 10^{12}$ vg/ml.

A temperature gradient during the PCR annealing/extension step was performed as a first step in transitioning a qPCR assay to a ddPCR assay. Data were collected from a ddPCR experiment following the Takara qPCR sample preparation protocol. The same upstream sample preparation and qPCR primers were used in order to directly compare temperature effects on assay performance between the two methods.

A. Standard Curve



B. Amplification

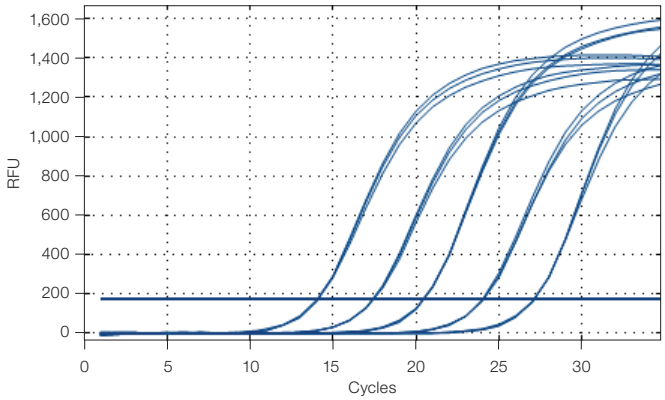


Fig. 1. qPCR data showing three replicates at each dilution for the standard curve (A) and an AAV2-CMV-GFP viral sample (B). The linear regression parameters for the standard curve are standard curve slope = –3.271, y-intercept = 36.949, R^2 = 0.999, and E = 102.2%. Cq, quantification cycle; RFU, relative fluorescence units.

The recommended ddPCR thermal cycling protocol was used instead of the qPCR thermal cycling protocol across a temperature gradient from 65 to 55°C during the annealing/extension phase. This is shown in Figure 2. Representative data from one of the standard curve dilutions (Figures 2A and 2B) and viral sample (Figures 2C and 2D) show that although the separation between positive and negative droplets varies with temperature in the 1-D plots (Figures 2A and 2C), the assay separation is robust across a wide range of temperatures run on the ddPCR platform. The separation increases as the annealing/extension temperature is decreased from 65 to 59°C and then remains constant down to 55°C. The concentration (Figures 2B and 2D) is relatively independent of temperature because an appropriate threshold can be defined that differentiates the negative and positive droplets. Often, choosing a temperature that creates the largest separation between positives and negatives is desired for ease of setting thresholds and to lessen the impact of sample matrix effects. In addition to the plot showing the individual concentrations for each temperature (Figures 2B and 2D), data from all temperatures

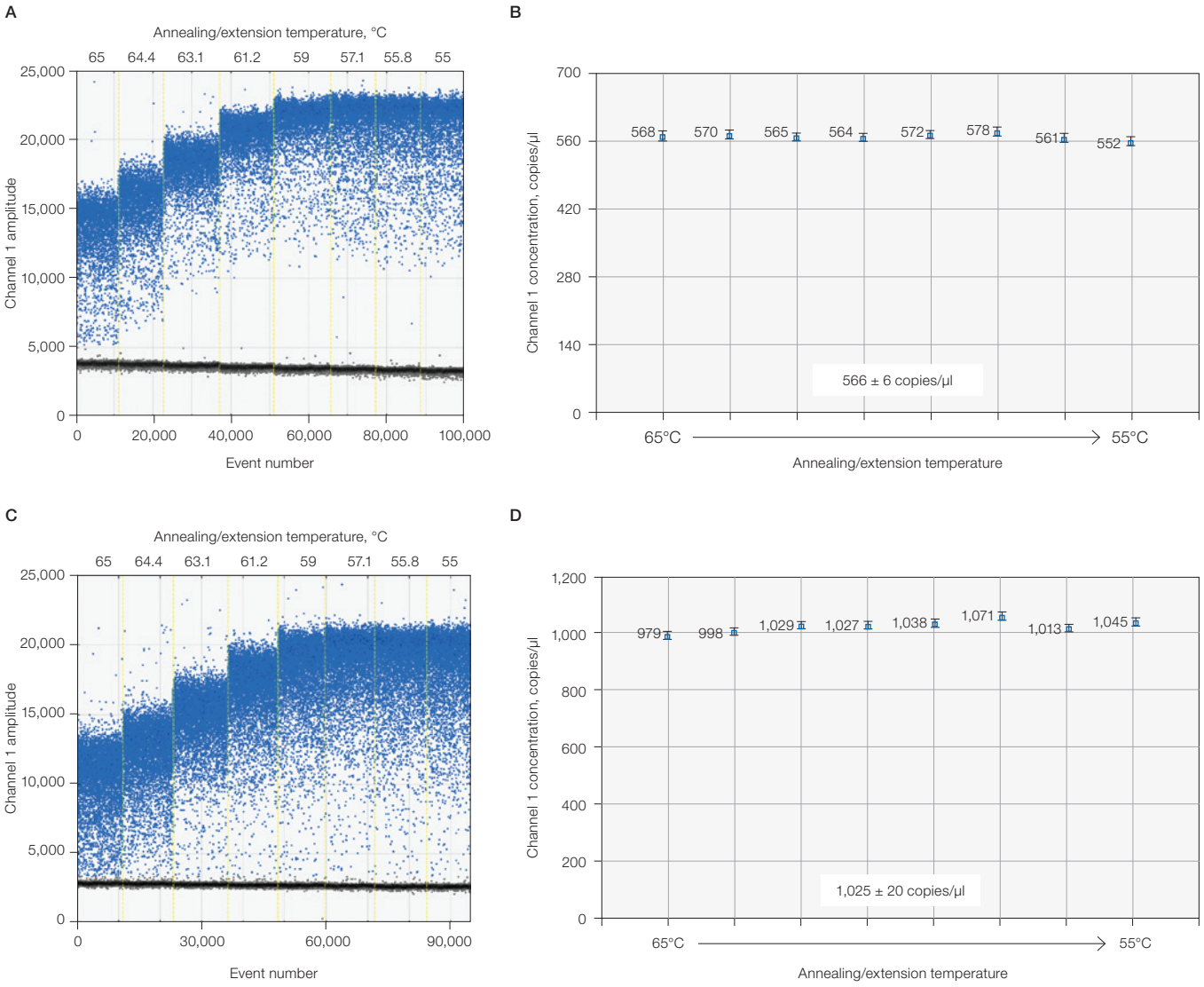


Fig. 2. Annealing/extension temperature gradient optimization data for Droplet Digital PCR using the standard curve dilution sample and a viral sample with the Takara kit primers and ddPCR EvaGreen® Supermix. Representative 1-D droplet plot data and concentration plot data are shown for a positive standard curve control sample (**A** and **B**) and an AAV2-CMV-GFP viral sample (**C** and **D**) that was thermal cycled with an annealing/extension temperature varying from 65–55°C (left to right). The separation between the positive and negative droplets varies with temperature in the 1-D plots (**A** and **C**) but the concentration (**B** and **D**) is independent of temperature because there is adequate separation between the positive and negative droplets. The data from all temperatures were merged and used to calculate the concentrations in the ddPCR reactions and 95% confidence intervals for the standard curve (**B**) and viral sample (**D**).

were merged and used to calculate the concentrations of the ddPCR reactions and 95% confidence interval for the positive control sample (Figures 2B, 566 ± 6 copies/µl) and the viral sample (Figure 2D, 1,025 ± 20 copies/µl), which is (4.15 ± 0.08) × 10^{12} vg/ml after correcting for sample dilutions. The concentration of the control sample was lower by a factor of 1.76 from the value assigned by the manufacturer for the standard curve. Because Droplet Digital PCR counts the number of physical amplicons present and does not use an ultraviolet (UV) measurement to assign concentrations, it can differ from the qPCR results. For example, Figure 3 (precolumn) shows that the concentration of a purified plasmid determined by Droplet Digital PCR can be about 55% lower than the expected concentration based on a UV measurement. The difference between the two measurements can be decreased to 10% or less by an additional column purification step (Figure 3, postcolumn).

Another common method for going from quantitative PCR to Droplet Digital PCR is optimizing the protocol for full access to the DNA. Adding a restriction enzyme that cuts nearby but not within the amplicon can increase amplicon accessibility. In addition to improving PCR, using a restriction enzyme in Droplet Digital PCR can separate two DNA targets that are physically located on the same DNA so that they separate into individual droplets to be counted separately.

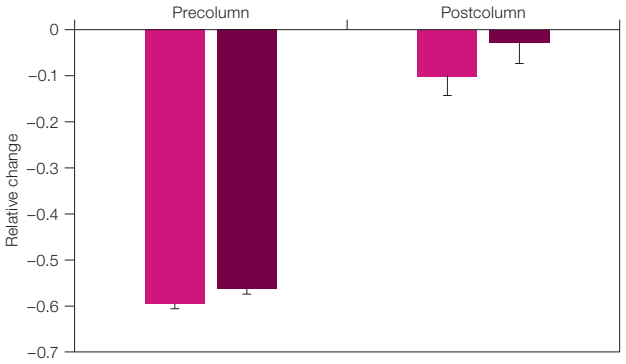


Fig. 3. Relative change between the copy number calculated from absorbance measurements and ddPCR concentration. The copy numbers were calculated using 660 Da/bp and a multiplicative factor of either 50 or 46.25. The ddPCR concentration was lower than expected relative to the calculated copy number for a plasmid before column purification. The agreement between the calculated and experimental copy number was much better after an additional small-scale column purification of the plasmid. ■ 50 ng x (cm/µl); ■ 46.25 ng x (cm/µl).

Figure 4 shows the effect of restriction enzyme digestion on the positive control from the qPCR kit and the AAV2-CMV-GFP virus. Each sample type was evaluated by including 5 U MspI per reaction in the qPCR and ddPCR reaction mix prior to thermal cycling. The restriction enzyme had different effects on the control material and the viral material for qPCR. The positive control from the AAVpro Titration Kit had minimal sensitivity to MspI, demonstrated by Cq values that were similar for the different dilutions with MspI and with no enzyme added (Figure 4A). However, the Cq values for the different dilutions of the viral prep were lower with MspI compared with the no-enzyme values (Figure 4B). The efficiency (103.7%) and R^2 value (0.999) were similar to the values without enzyme and within acceptable parameter ranges. The concentrations determined by Droplet Digital PCR for one dilution of the positive control at different annealing/extension temperatures with no enzyme (566 ± 6 copies/µl) and with MspI (557 ± 9 copies/µl) did not change (Figure 4C). In contrast, the viral AAV2-CMV-GFP sample parameters varied with MspI and showed the expected approximate twofold increase in ITR concentration only when the ITR regions were separated by a restriction enzyme. The Cq values for the different dilutions of the viral prep were lower with MspI compared with the no-enzyme values (Figure 4B) and the ddPCR concentrations increased from 1,025 ± 20 copies/µl with no enzyme to 1,864 ± 17 copies/µl with MspI in the ddPCR reaction mix (Figure 4D). This was true at each of the different annealing/extension temperatures. The differential effect of MspI on the control sample and viral sample suggests that the two samples may have different template accessibility and could introduce a quantification error using qPCR, which assumes that the standard and template have similar amplification. The decrease in Cq values and increase in ddPCR copy number using MspI resulted in ITR concentrations of (1.33 ± 0.25) × 10^{13} copies/ml for quantitative PCR and (7.35 ± 0.15) × 10^{12} copies/ml for Droplet Digital PCR. Figure 5 shows the viral genome copies/µl in the stock sample, which were calculated with the qPCR

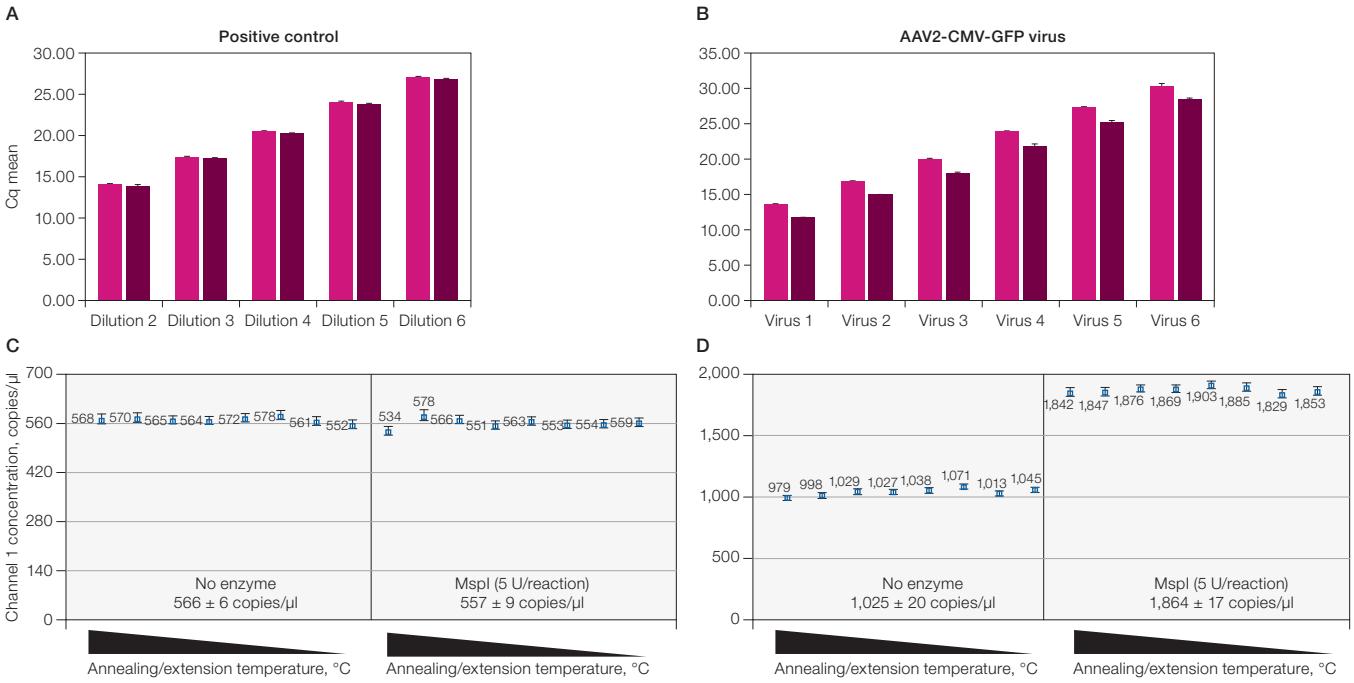


Fig. 4. Effect of restriction enzyme digestion. MspI (5 U per reaction) was added to a control (**A** and **C**) and an AAV2-CMV-GFP (**B** and **D**) PCR sample prior to thermal cycling. MspI has minimal effect on qPCR Cq values (**A**) or ddPCR concentrations (**C**) for the positive control. MspI lowers the qPCR Cq values (**B**) and increases the ddPCR concentration for the AAV2-CMV-GFP virus (**D**). The data from all temperatures were merged and used to calculate the concentrations in the ddPCR reactions and 95% confidence intervals for the positive control (**C**) and viral sample (**D**). The no-enzyme data (**C** and **D**) are reproduced from Figures 2B and 2D. ■ No enzyme; ■ MspI. Cq, quantification cycle.

data from the Takara protocol and with a separate viral titration using ddPCR data from the Bio-Rad protocol. The final viral titer concentrations in copies/µl for the two protocols were (3.98 ± 0.77) × 10^9 for quantitative PCR and (8.72 ± 0.10) × 10^9 for Droplet Digital PCR. The titer of the AAV-CMV-GFP stock sample from Vigene Biosciences, expressed in gene copies, was 2.17 × 10^{13} gc/ml or 2.17 x 10^{10} gc/µl.

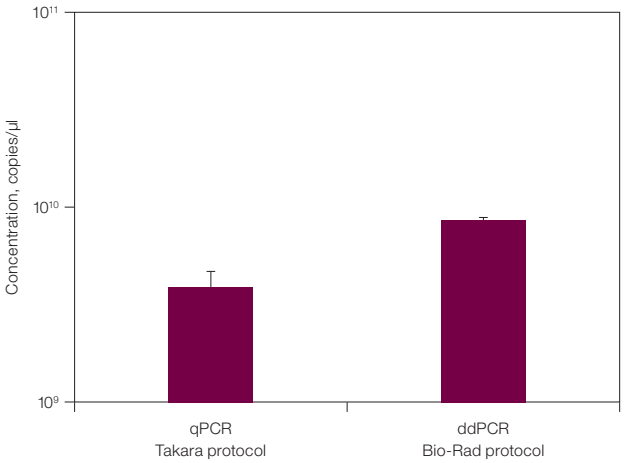


Fig. 5. Final viral genome concentrations calculated from the qPCR and ddPCR protocols. The concentrations in copies/µl of viral genomes for the two protocols were (3.98 ± 0.77) × 10^9 (qPCR protocol, Takara) and (8.72 ± 0.10) × 10^9 (ddPCR protocol, Bio-Rad).

Discussion

The primary focus of this study was to discuss how to bridge qPCR and ddPCR data when transitioning from one method to the other. The data we show and discuss is meant to aid in understanding the process. Ultimately, once the method and protocol have been developed, comparability between the two methods is robust but not identical. As ddPCR assays are often run without prequantified standards and only with a positive and negative control, it is important to ensure full amplification of the DNA. For this reason, we show several common methods used to ensure a robust transition into Droplet Digital PCR. A temperature gradient and addition of a restriction enzyme that cuts outside the amplicon(s) are two common optimizations recommended when moving an assay into Droplet Digital PCR.

Quantitative PCR and Droplet Digital PCR have similarities, including exponential amplification and fluorescent chemistries (DNA-binding dyes or hydrolysis probes) for the detection and quantification of nucleic acids. It is important to understand the differences when transitioning from one method to the other. One significant difference is that Droplet Digital PCR uses the fraction of negative droplets and Poisson statistics to determine the absolute number of amplifiable copies/µl of reaction and the corresponding confidence intervals while qPCR requires a standard curve for quantification. As a result, one of the primary reasons for final concentration differences between the two technologies is the standard curve used to quantify the sample in qPCR. This measurement of DNA concentration is assigned based on the slope and y-intercept derived from a linear regression analysis of the standard curve. The original standard curve quantification most commonly comes from a UV measurement, which can be inaccurate due to impurities and other sample artifacts. For this reason, we explored the UV measurement as a common source of variation between qPCR and ddPCR quantification. Furthermore, qPCR data analysis assumes that the PCR reactions of the standard curve samples and unknown samples have identical amplification efficiencies that are constant for every thermal cycle required to reach the quantification cycle. Here we show that the standard curve material did not appear to be similar, in that the standard curve and the viral genome responded differently to the addition of the restriction enzyme (Figure 4).

The concentration of the standard must be determined by an independent technique. A common technique for standard quantification is to measure the A_{260} value with a spectrophotometer and calculate the copy number using the number of base pairs in the standard and an average molecular weight for a DNA base pair. The A_{260} value is not specific for DNA and is affected by nucleic acid structure due to the hyperchromicity of single-stranded DNA compared with double-stranded DNA. In addition, organic contaminants, protein, or residual *E. coli* DNA in the standard material could affect the A_{260} value. The difference in concentration between a specific ddPCR measurement and a nonspecific UV absorbance measurement that detects any compound with an absorbance at 260 nm has been published (Lock et al. 2014). We show that these concentration differences may be minimized by an additional column purification step (Figure 3). In addition, assumptions made during the conversion of absorbance to concentration can also cause the ddPCR copy number to differ from the concentration calculated from a UV absorbance measurement. Some of these assumptions are the molecular weight (660 Da/bp, 650 Da/bp, or a de novo value based on the individual base composition), the absorbance-to-concentration conversion factor (50 or 46.25 ng x [cm/µl]), that the DNA is completely pure with no extraneous host cell DNA, and that the DNA topology is homogenously double stranded.

Fluorescence detection of DNA is an alternative method for determining DNA concentration relative to a control sample. Fluorescence assays can be specific for single-stranded or double-stranded DNA but temperature fluctuations and any miscellaneous double-stranded DNA can influence the accuracy of the assays. In addition, the DNA structure, topology (supercoiled, relaxed, or linear), and sequence effects can affect DNA intercalating dyes. All of these possible causes of standard curve quantification variation will result in some differences in actual quantification when transitioning to Droplet Digital PCR.

It was mentioned that differential PCR efficiencies between the standards and unknown samples can influence qPCR results for quantification based on a standard curve. Data shown in Figure 4 demonstrate how a standard curve sample does not match the sample's PCR accessibility. If both samples had the same primer accessibility, the same concentration of standard and unknown would have the same Cq values. We used endonuclease restriction digestion with MspI to modify the template accessibility. Our qPCR data (Figures 4A and 4B) show that template access for the positive control samples is unaffected by restriction digest with MspI because there is no change in Cq values, but Cq values for the viral template decrease with MspI treatment due to an increased accessibility of the viral template. This differential template access will affect quantification accuracy when the sample conditions are different from the standard curve sample. A similar effect of MspI was seen for the ddPCR data (Figures 4C and 4D). In the case of a template with two ITRs, the increase in concentration seen for the viral sample using Droplet Digital PCR is a combination of template accessibility and linkage where the two ITRs are physically linked on the same piece of DNA. If the ITRs are not separated, they would be counted once per viral genome.

A general recommended method to translate a qPCR assay to a ddPCR assay would consist of comparing the results of a standard curve dilution and a realistic sample using similar conditions. Droplet Digital PCR, as a method for physical counting of amplicons, should be optimized for PCR efficiency using a temperature gradient and a restriction enzyme when possible. In the AAV example (Figure 5), an additional optimization of the upstream processing of the viral ddPCR sample was essential. Both protocols begin by digesting unencapsidated nucleic acids using DNase I, followed by splitting the DNase-treated sample into two aliquots for downstream parallel sample processing using qPCR and ddPCR protocols. The qPCR protocol is the current working protocol. The ddPCR protocol involves a tenfold serial dilution of the DNase-treated virus using polyA, lysis of the diluted virus at 95°C for 10 min, and assembling PCR reactions containing 5 U of MspI and less than 5,000 copies/µl of template. The thermal cycling protocol includes a temperature gradient from 5°C below to 5°C above the annealing/extension temperature used for qPCR. The conversion factor can then be calculated from the ratio of titers determined by the two protocols. In this case, the qPCR titer can be converted to the ddPCR titer by multiplying it by 2.19 (that is, the ratio of ddPCR titer with MspI to qPCR titer with a standard protocol, or 8.72/3.98). Testing across a dynamic range of input concentrations is recommended to evaluate the sensitivity and consistency of the qPCR to ddPCR conversion factor. MspI was chosen for this experiment because of our previous experience with ITR assays, but 5 U of any restriction endonuclease (HaeIII, MseI, AluI, HindIII, or CviQI) that does not have a recognition site within the amplicon should be included in the ddPCR protocol to improve template accessibility for a region (promoter, enhancer, polyA signal sequence, gene of interest, etc.) outside of the ITR.

Ultimately, there may be a combination of process-related and experimental factors contributing to the differences in viral titers between qPCR and ddPCR platforms.

Conclusion

With qPCR, it is known that there are large differences between laboratories. For example, recombinant adeno-associated virus reference standard material (AAV2 serotype and AAV8 pseudotype) was distributed to 16 laboratories worldwide and there was a variation in interlaboratory precision and accuracy despite attempts to standardize the assays by providing detailed protocols and common reagents (Lock et al. 2010 and Ayuso et al. 2014). This can be overcome by transitioning assays from quantitative PCR to Droplet Digital PCR, which has very small day-to-day and lab-to-lab variation. A simple bridging study can directly compare consistent and robust ddPCR results to past qPCR data.

References

Ayuso E et al. (2014). Manufacturing and characterization of a recombinant adeno-associated virus type 8 reference standard material. Hum Gene Ther 25, 977–987.

Lock M et al. (2010). Characterization of a recombinant adeno-associated virus type 2 reference standard material. Hum Gene Ther 21, 1,273–1,285.

Lock M et al. (2014). Absolute determination of single-stranded and self-complementary adeno-associated viral vector genome titers by droplet digital PCR. Hum Gene Ther Methods 25, 115–125.

Nwokeoji AO et al. (2017). Accurate quantification of nucleic acids using hypochromicity measurements in conjunction with UV spectrophotometry. Anal Chem 89, 13,567–13,574.

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