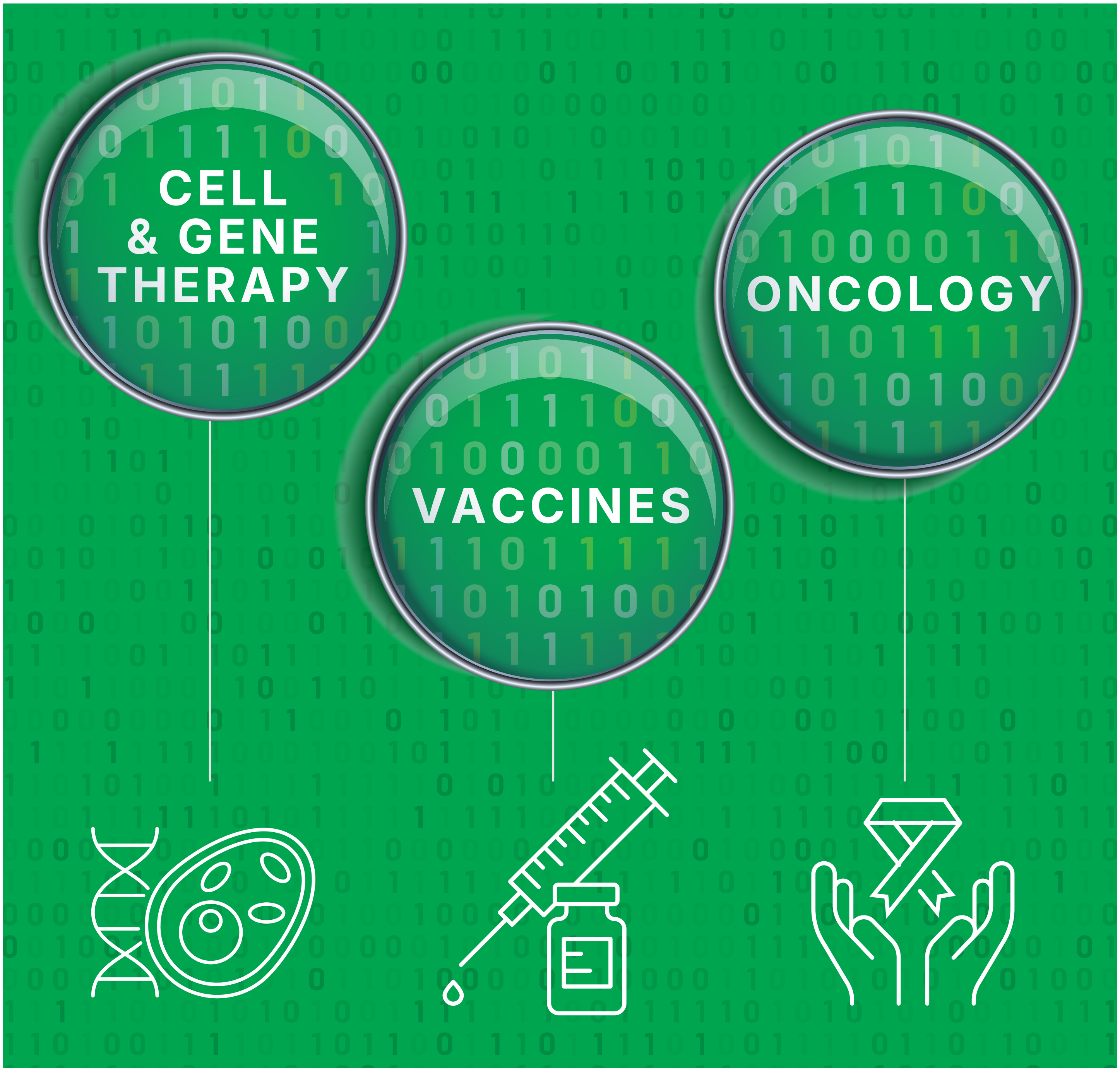
eBOOK

Past, Present and Future of Digital PCR



Visit **bio-rad.com/dropletdigitalpcr** for more information.

Table of Contents

© 2025 Bio-Rad Laboratories, Inc.

Foreword

Digital PCR (dPCR) is relied upon by researchers and clinicians across the world for nucleic acid quantification. In particular, the rise of Droplet Digital™ PCR (ddPCR™) has solidified dPCR as a competitive solution providing increased sensitivity and precision.

Advances in modern medicine now offer the opportunity to treat diseases previously thought to be incurable. From cell and gene therapy development to cutting-edge cancer diagnostics, PCR solutions with superior analytical capabilities are in high demand. Where quantitative real-time PCR (qPCR) has previously been the standard method, digital PCR has developed into a reliable way to achieve absolute quantification of nucleic acids. In addition, the sensitivity offered by ddPCR presents a significant advantage for detecting low-abundance targets, even in complex sample matrices.

As a result, ddPCR has become an essential tool in many fields. By overcoming the bias and variability concerns associated with relative quantification, ddPCR is well-suited for quality control during therapeutics development – ensuring the safety and efficacy of transformative cell and gene therapies and vaccines. In cancer biomarker analysis, ddPCR technology is a rapid and precise tool to analyze circulating tumor DNA, harnessing the full potential of liquid biopsies.

This eBook explores the past, present and future of digital PCR, from its humble beginnings to its establishment as a method trusted by researchers worldwide. We discuss the analytical capabilities that make ddPCR so versatile as well as the emerging applications of this technique in gene and cell therapy, cancer biomarkers analysis and vaccine development.

© 2025 Bio-Rad Laboratories, Inc.

The Evolution of Droplet Digital PCR Technology

The rediscovery of dPCR

Researchers use polymerase chain reaction (PCR) to amplify specific nucleic acid sequences. Over the last 40 years, scientists have developed a wide range of novel PCR methods, which can provide in-depth qualitative and quantitative information. One such variation is quantitative real-time PCR (qPCR). qPCR can determine the approximate number of amplified molecules in a sample over time, but it requires comparison of the sample to a standard curve. In comparison, digital PCR (dPCR) does not require a standard curve. Instead, an instrument subdivides each sample into thousands of partitions, each of which function as an individual PCR reaction. After amplification, each partition is checked for fluorescently labeled DNA products and software applies Poisson statistical analysis to quantify the absolute number of target molecules in the sample.

The first description of dPCR – in which Saiki and colleagues detected a single β-globin gene within 500,000 cells – was published in 1988, predating qPCR.[1] However, it was largely abandoned in favor of qPCR until partitioning capabilities improved, as dPCR was considered laborious and costly, with limited usage (Figure 1).[2] In 2006, dPCR gained new popularity with the creation of microfluidic chips by Fluidigm, and the creation of the first chip-based dPCR (cdPCR) platform. Then, in 2010, Quantalife (later acquired by Bio-Rad) developed the first Droplet Digital PCR (ddPCR) platform.[3]

Based on oil–water emulsions, the ddPCR instrument partitions samples by generating 20,000 equally sized droplets containing 0–1 template molecules. This enables independent target amplification which the instrument measures by passing the droplets through a fluorescence detector. dPCR can now be used for a wide variety of applications, including identifying copy number variants, quantifying reference materials and analyzing liquid biopsies.

Exploring the dPCR publication guidelines

Throughout its range of applications, reproducibility and reliability of dPCR results is essential. To this end, Huggett et al. published the minimum information for publication of quantitative digital PCR experiments (dMIQE).[4,5] These guidelines state the essential information that researchers must include in their publications about their dPCR experiments, to avoid publishing low-quality and irreproducible data. As dPCR technology advanced and became more popular, so too did the dMIQE guidelines, which were then updated in 2020.

The information needed for the dMIQE falls under three categories: sample preparation, dPCR protocol and data analysis.

Sample preparation

Nucleic acid isolation and processing affect the accuracy of dPCR experiments. Using a range of different methods, such as enzymatic digestion and sonication, nucleic acids can be extracted from a range of different specimens, including clinical samples, animal models and cultured cells. dPCR sensitivity can be improved by specific sample processing methods such as restriction endonuclease-driven DNA cleavage or DNA denaturing. However, the sample processing methods must be considered during data analysis for accurate results. For example, DNA denaturation produces

© 2025 Bio-Rad Laboratories, Inc.

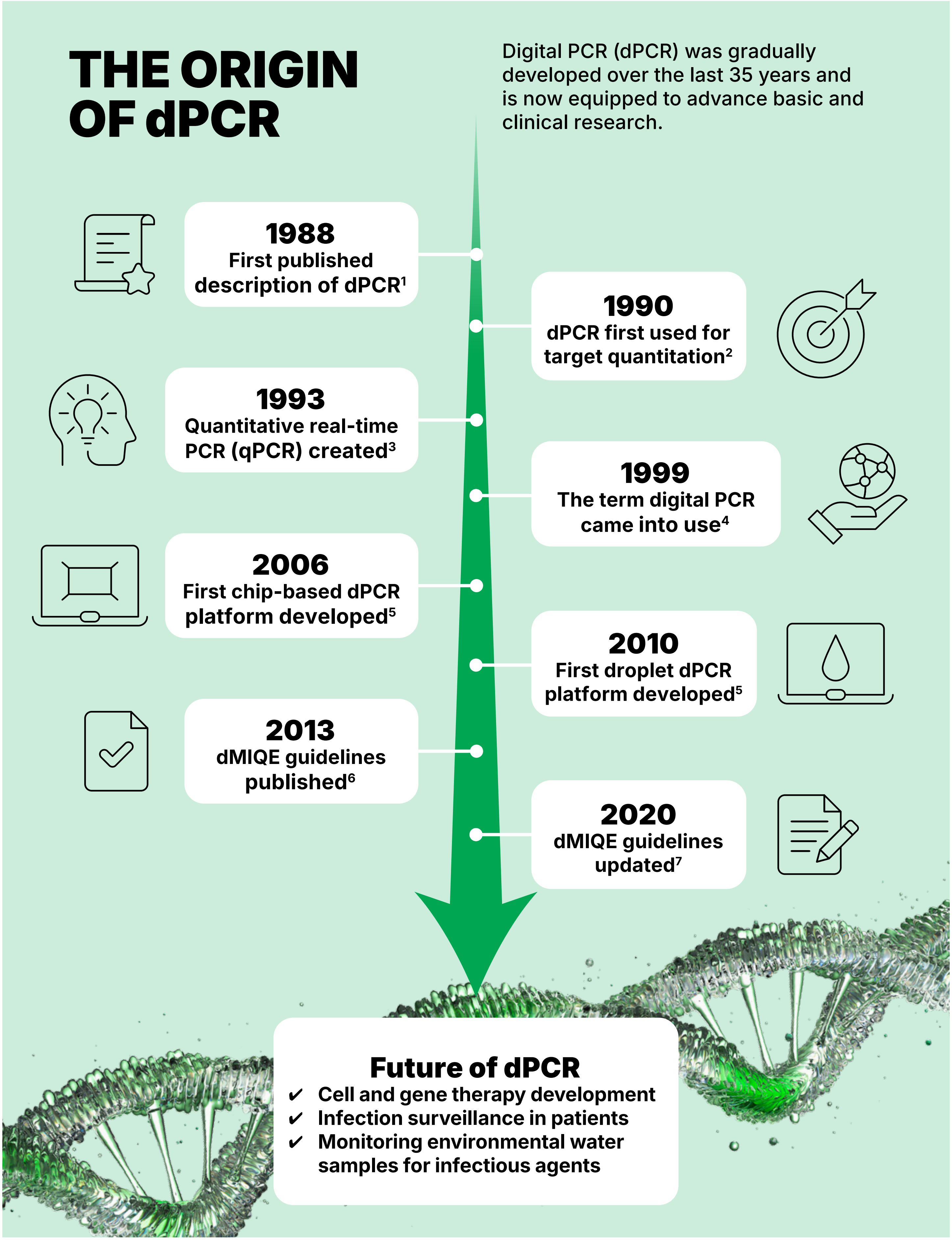


Figure 1. Notable events in the development of dPCR.

two template molecules, which can lead to an overestimation in sequence quantity if not accounted for. If RNA is extracted, the efficiency of RNA transcription to DNA will also affect quantitation of target frequency. Therefore, information about the reverse transcription step – such as RNA quantity, reagents used and protocols employed – is essential for reproducibility. The quality of extracted DNA must also be assessed, as damaged nucleic acids can impair target amplification and quantity.

dPCR protocol

Detailed information about pre-reaction mixture preparation and the instrument used is essential for reproducibility, as the chosen procedure can influence target amplification. The dPCR protocol should be optimized to ensure that primers are not dimerizing, and to find the best primer and probe concentrations. Controls are key for reproducibility and high-quality data, to assess if inhibitors are affecting amplification and ensure that samples are not contaminated. Positive controls should comprise primers, probes and target sequence-containing DNA, while negative controls should contain primers, probes but no template molecules. Experimental variation should be monitored using biological replicates and prepared using identical sample processing procedures.

Data analysis

Finally, details about data analysis are also required for the dMIQE. Example plots of positive and negative controls should be provided to demonstrate assay success. In addition, positive controls can be used to guide the setting of appropriate thresholds to separate negative partitions from positive partitions. Results from different biological replicates should be compared to assess variation and estimate the level of random error in the target frequency quantifications. Overall, research and researchers that follow the dMIQE guidelines ensure that their dPCR experiments are designed correctly, their results are reproducible and their data are comparable to other studies.

High-level precision across a range of applications

Ensuring that dPCR studies are reproducible and robust is essential as the technique becomes increasingly used across a wide range of fields in both preclinical and clinical settings, such as cell and gene therapies (CGT), vaccine development and oncology. These contexts demand extremely high levels of quality control and accuracy, and qPCR has typically been the go-to method for measuring levels of genetic material. However, dPCR offers several advantages over qPCR that are now being explored.

ddPCR technology has been shown to give less variable and more precise results than qPCR when quantifying viral vector titres in CGT development.[6,7] As the buffers required for DNA purification can contain inhibitors that impair PCR amplification, ddPCR can be more tolerant of inhibitors than qPCR. In addition, ddPCR solutions have been shown to have greater reproducibility and sensitivity at low copy numbers than qPCR.[8] ddPCR technology enables absolute quantification of nucleic acids without requiring a standard curve, even at very low levels, which is an advantage in biomarker analysis and vaccine manufacturing.[9,10]

After decades of being overlooked in favour of qPCR, dPCR – and in particular ddPCR – is now re-emerging as a competitive, reliable technique for quantifying genetic material. ddPCR technology provides researchers with increased sensitivity, greater precision and enhanced inhibitor tolerance, and it is therefore rapidly becoming an important technique to evaluate genetic material in a variety of different fields. However, it is essential for these techniques to be performed and reported in line with the dMIQE guidelines to ensure accurate, reproducible and high-quality results.[11] This eBook explores the emerging applications of ddPCR solutions, and how it is transforming several different fields, including CGT research, vaccine development and cancer biomarker analysis to become a trusted method for researchers around the world.

References

1. Morley AA. Digital PCR: A brief history. *Biomol Detect Quantif*. 2014;1(1):1–2. doi: 10.1016/j.bdq.2014.06.001
2. Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*. 1988;239(4839):487–491. doi: 10.1126/science.2448875
3. Baker M. Digital PCR hits its stride. *Nat Methods*. 2012;9(6):541–544. doi: 10.1038/nmeth.2027
4. Huggett JF, Foy CA, Benes V, et al. The digital MIQE guidelines: Minimum information for publication of quantitative digital PCR experiments. *Clin Chem*. 2013;59(6):892–902. doi: 10.1373/clinchem.2013.206375
5. The dMIQE Group, Huggett JF. The digital MIQE guidelines update: Minimum information for publication of quantitative digital PCR experiments for 2020. *Clin Chem*. 2020;66(8):1012–1029. doi: 10.1093/clinchem/hvaa125
6. Lin H-T, Okumura T, Yatsuda Y et al. Application of droplet digital PCR for estimating vector copy number states in stem cell gene therapy. *Hum Gene Ther Methods*. 2016;27(5):197–208. doi: 10.1089/hgtb.2016.059
7. Dobnik D, Kogovšek P, Jakomin T, et al. Accurate quantification and characterization of adeno-associated viral vectors. *Front Microbiol*. 2019;10:1570. doi:10.3389/fmicb.2019.01570
8. Lou Y, Chen C, Long X, et al. Detection and quantification of chimeric antigen receptor transgene copy number by droplet digital PCR versus real-time PCR. *J Mol Diagn.* 2020;22(5):699–707. doi: 10.1016/j.jmoldx.2020.02.007
9. Abachin E, Convers S, Falque S, Esson R, Mallet L, Nougarede N. Comparison of reverse-transcriptase qPCR and droplet digital PCR for the quantification of dengue virus nucleic acid. *Biologicals*. 2018;52:49–50. doi: 10.1016/j.biologicals.2018.01.001
10. Gezer U, Bronkhorst AJ, Holdenrieder S. The clinical utility of droplet digital PCR for profiling circulating tumor DNA in breast cancer patients. *Diagnostics*. 2022;12(12):3042. doi: 10.3390/diagnostics12123042
11. Murphy LA, Marians RC, Miller K, et al. Digital polymerase chain reaction strategies for accurate and precise detection of vector copy number in chimeric antigen receptor T-cell products. *Cytotherapy*. 2023;25(1):94–102. doi: 10.1016/j.jcyt.2022.09.004

© 2025 Bio-Rad Laboratories, Inc.

Blueprints of a Trusted Gold Standard: ddPCR Technology in Research and Development

After decades of being overlooked, digital PCR – particularly Droplet Digital™ PCR (ddPCR™) – is now recognized as a robust, accurate and reliable technique. In many areas of research, such as cell and gene therapy, vaccines and cancer biomarkers, ddPCR technology offers multiple advantages over other methods. Managing the safety and efficacy of complex biopharmaceutical therapies and novel vaccines can be a challenging and time-consuming process. In oncology research, longitudinal biomarker monitoring requires rapid turnarounds and minimally invasive sampling methods. In all these fields – from initial discovery stages, through preclinical and clinical testing, to manufacturing approved products – ddPCR technology has emerged as a new gold standard method to respond to a myriad of workflow challenges.

Trusted in cell and gene therapy development

From initial testing, through quality control and assessment into clinical research, ddPCR solutions can support:

- CMC submissions
- Reliable assessment of purity, efficacy and safety
- Efficient and scalable process development
- Effective evaluation of therapies in patients

Applications

- Analyze plasmid integrity
- Determine viral titer and empty–full capsid ratio
- Quantify transgene copy number and expression
- Detect mycoplasma and residual DNA
- Determine biodistribution
- Evaluate dose response

Trusted during vaccine development

From initial antigen characterization, through manufacture and into preclinical and clinical studies, ddPCR solutions can help facilitate:

- Tracking emerging variants of evolving pathogens
- Optimal cell line development
- Quality control in manufacturing
- Meeting regulatory guidelines
- Effective evaluation of efficacy in clinical studies

Applications

- Discriminate between highly specific strains and variants
- Identify stable and highly expressing cell lines
- Quantify transgene copy number and expression
- Quantify viral load and determine integrity
- Detect residual host DNA contamination
- Evaluate dose response

Trusted for biomarker detection and analysis

Due to its rapid turnaround, low costs and minimal, non-invasive sample requirements, ddPCR solutions are used extensively in oncology research to analyze circulating tumor DNA, enabling:

- Detection of predefined biomarkers
- Post-treatment monitoring
- Prediction of treatment outcome and relapse
- More adaptive study designs
- Development of personalized medicine

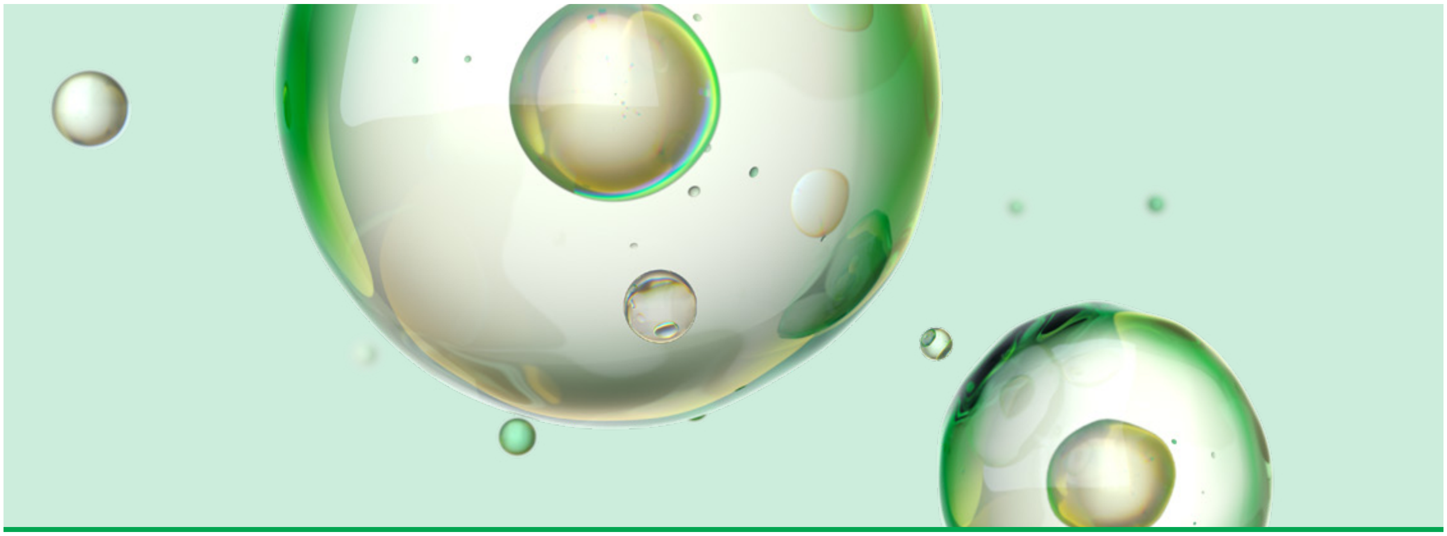
Applications

- Assess rare genetic variants
- Determine treatment effectiveness
- Identify molecular residual disease cells post-treatment

Key benefits of ddPCR technology

- Precision
- Accuracy
- Inhibitor tolerance
- Multiplexing
- Time to results
- Sensitivity/specificity

Click here to discover end-to-end solutions for your workflow



Advancing Quality Control in Cell and Gene Therapy Development With ddPCR technology

The landscape of modern medicine is being transformed by the rapid advancement of cell and gene therapies (CGTs), which offer unprecedented opportunities to treat previously incurable diseases. With over 4,000 clinical trials and 139 clinically approved products globally, these therapies are rapidly progressing from experimental concepts to clinical reality.[1] However, with this remarkable progress comes the critical need for precise, accurate and reliable quality control methods throughout the development and manufacturing processes. Droplet Digital PCR (ddPCR) has emerged as a pivotal technology in this space, offering superior analytical capabilities that are essential for ensuring the safety and efficacy of these complex therapeutic products.

Cell and gene therapy in a nutshell

The journey of CGT began in 1928 when Frederick Griffith discovered that cells could change their properties by taking up exogenous genetic material.[2] Since then, the field has experienced both remarkable successes and significant setbacks, including the tragic death of Jesse Gelsinger in 1999, which highlighted the critical importance of safety in viral vector-based therapies.[2]

The therapeutic efficacy of CGT hinges on the precise delivery and expression of nucleic acids inside the human body. Gene therapy typically involves delivering exogenous DNA into patient cells using vectors (often viral vectors).[3] In cell therapy, the patient's cells or donor cells serve as the DNA delivery mechanism. These cells are extracted, modified *ex vivo*, expanded and reintroduced to the patient.[4] This approach eliminates many of the safety concerns associated with direct viral injection while enabling mass production of therapeutic cells.

The DNA being delivered can be manipulated through recombinant DNA technologies, allowing for the restoration of lost functions, the introduction of new capabilities or the modulation of existing cellular processes. The advent of advanced gene editing technologies, particularly CRISPR/Cas systems, has accelerated progress in this field.[5,6] Combined with improved viral vectors such as adeno-associated viruses (AAVs), which offer broad tissue tropism with minimal immunogenicity, these technologies have created new possibilities for treating a wide range of conditions from hereditary genetic disorders to cancer and cardiovascular disease.[7]

Yet, these therapies are complex and must be evaluated not just for their biological activity, but also for their safety, stability and manufacturing reproducibility.

The critical role of quality control in CGTs

As cell and gene therapies move from laboratory bench to clinical application, the complexity of manufacturing and quality control has become increasingly apparent. These therapies often work with patient or donor cells that are

© 2025 Bio-Rad Laboratories, Inc.

inherently heterogeneous and complex, making consistency and reproducibility challenging. Moreover, current manufacturing processes are largely manual, relying heavily on operator experience and judgment, which can result in considerable batch-to-batch variation.

Hence, quality control plays an essential role throughout the CGT development pipeline, from initial research and development through to clinical trials and commercial manufacturing. Researchers must evaluate multiple critical parameters, including viral vector integrity, purity, concentration and the absence of contaminants. For cell-based therapies, additional considerations include transgene copy number, expression levels and cellular viability. The precision and accuracy of these measurements directly impact patient safety, dosage determination and treatment efficacy.

Traditional quantitative real-time PCR (qPCR) has been the standard method for many of these quality control tests. However, qPCR relies on standard curves and reference materials for quantification, which can introduce variability and bias into the results. The technique is also susceptible to inhibitors commonly used in purification buffers and other manufacturing reagents, potentially leading to inaccurate measurements. The advent of Droplet Digital PCR has allowed researchers to address many of these limitations (Table 1).[8,9,10,11] The ddPCR solution provides absolute quantification without requiring standard curves or reference materials, eliminating a major source of variability and bias. The technique also enhances sensitivity, allowing detection of rare targets that might be missed by qPCR. Additionally, ddPCR technology demonstrates superior tolerance to inhibitors commonly encountered in CGT manufacturing processes, ensuring accurate measurements even in challenging sample matrices.

Table 1: Comparison of qPCR and ddPCR technology characteristics.

Feature	qPCR	ddPCR Technology
Quantification	Relative (requires standard curve)	Absolute (no standard curve)
Sensitivity	Moderate	High (can detect single copies)
Inhibitor tolerance	Low	High
Reproducibility	Variable	High
Setup complexity	Complex calibration	Streamlined, fewer controls

ddPCR applications across the CGT pipeline

The use of ddPCR solutions extend across multiple stages of the CGT product lifecycle, offering critical analytical capabilities from early research through commercial manufacturing and clinical monitoring (Figure 1).

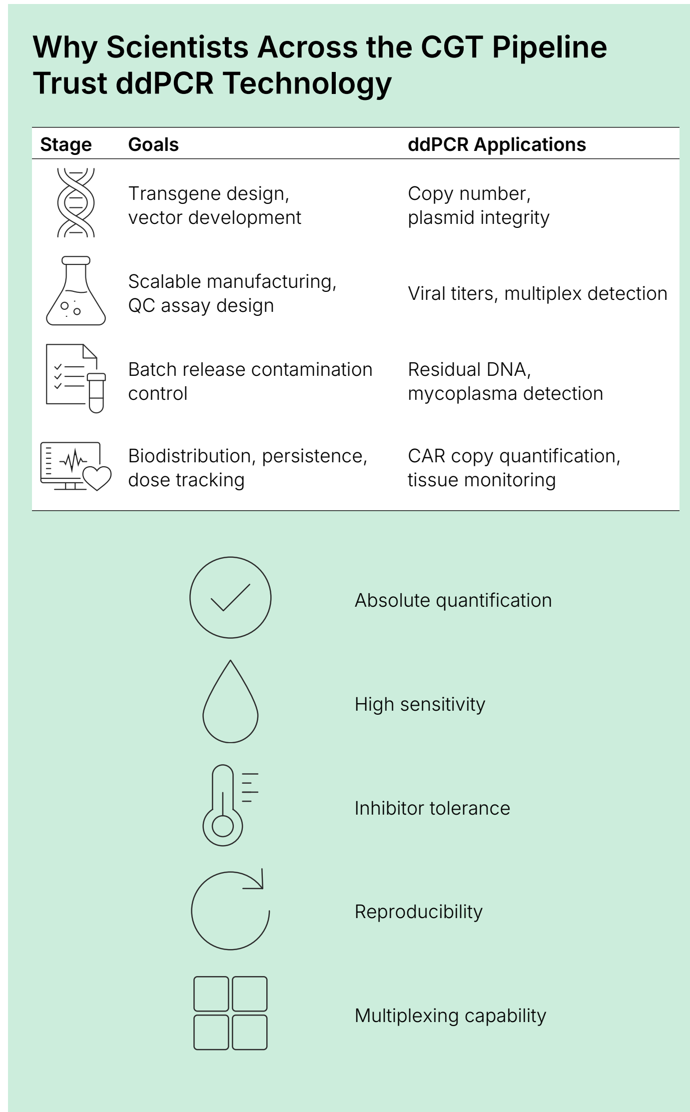


Figure 1. ddPCR technology application throughout the CGT product lifecycle.

© 2025 Bio-Rad Laboratories, Inc.

Research and development

In early-stage research, ddPCR solutions facilitate the characterization of gene delivery vectors and engineered cells. This capability is essential for optimizing transfection or transduction protocols and ensuring consistent gene transfer efficiency. Understanding how many copies of a transgene are incorporated and whether they are stably expressed is central to characterizing CGT products. Droplet Digital PCR provides an accurate, reproducible measurement of transgene copy number, even at low abundance or in highly variable samples such as patient-derived cells. Researchers have demonstrated that ddPCR technology provides greater precision and reproducibility compared to qPCR when quantifying vector copy numbers in transduced cells, leading to more reliable results.[10]

Process development and manufacturing

In manufacturing settings, ddPCR solutions serve multiple quality control functions. The technology enables manufacturers to precisely quantify vector titer, assess integrity and confirm purity, which is critical for ensuring consistent potency across production batches.[12] ddPCR technology excels at detecting low copy number sequences, verifying the presence or absence of contaminants and measuring residual DNA in final products. Moreover, unlike qPCR, which can be affected by the various buffers and reagents used in purification processes, ddPCR technology maintains accuracy even in the presence of potential inhibitors.[11] This reliability is particularly important during process development, where manufacturers need to evaluate the impact of different purification methods on product quality.

For cell-based therapies, ddPCR solutions allow for the precise monitoring of transgene expression levels and copy number throughout the manufacturing process. This capability is especially valuable for CAR-T cell therapies, where the level of chimeric antigen receptor expression directly impacts therapeutic efficacy. Studies have shown that ddPCR technology can detect CAR copies at lower concentrations than qPCR while providing superior repeatability and reproducibility.[9,13]

Quality control and regulatory compliance

The reproducibility, accuracy and speed of ddPCR solutions make it particularly well-suited for good manufacturing practice (GMP) settings and the stringent regulatory expectations for CGT quality control. The technology's ability to provide absolute quantification without reliance on standard curves reduces measurement uncertainty and supports more robust regulatory submissions. Additionally, its enhanced precision helps reduce the number of replicates required for statistical significance, potentially streamlining analytical workflows and reducing costs. ddPCR technology plays a vital role in batch release, potency and purity testing. The development of ddPCR assays for specific CGT applications, such as the detection of mycoplasma contamination in cell cultures, demonstrates the potential of the technique for addressing unique quality control challenges in this field.

Preclinical and clinical studies

In clinical settings, ddPCR technology supports both safety monitoring and efficacy assessment. ddPCR solutions enable precise tracking of therapeutic cells in patient samples, providing insights into biodistribution, persistence and potential off-target effects.[14] This monitoring is essential for dose optimization and safety assessment, particularly for novel therapies where long-term effects are not yet fully understood. The enhanced sensitivity of ddPCR technology also makes it valuable for detecting rare genetic variants or low-level transgene expression.

Future directions

As CGTs move toward personalized medicine paradigms, the demand for granular, high-fidelity molecular analysis tools will only increase. Droplet Digital PCR is already expanding its role from endpoint testing to integrated process analytics thanks to the added flexibility of multiplexing. Moreover, ddPCR's compatibility with single-cell and low-input workflows makes it well-suited for future advancements, including single-cell profiling of gene-modified cells, integration with AI-driven manufacturing systems and in-process biosensor feedback in automated cell culture environments.[15]

As regulatory agencies continue to raise the bar on analytical validation, ddPCR's reproducibility and compliance with standards such as dMIQE (minimum information for publication of quantitative digital PCR experiments) position it as a trusted cornerstone of CGT development.[16]

The integration of ddPCR solutions into CGT workflows is more than just a technological upgrade; it represents a commitment to the highest standards of quality and safety that these life-changing therapies demand. As the field continues to evolve, ddPCR technology will play an increasingly important role in ensuring the safety and efficacy of these transformative therapies, ultimately contributing to better patient outcomes and the continued advancement of precision medicine.

Learn more about ddPCR applications in CGT here

© 2025 Bio-Rad Laboratories, Inc.

References

1. American Society of Gene and Cell Therapy, Citeline. The Gene, Cell, & RNA Therapy Landscape report. Q1 2025 Quarterly Data Report. https://www.asgct.org/global/documents/asgct-citeline-q1-2025-report.aspx
2. Wirth T, Parker N, Ylä-Herttuala S. History of gene therapy. *Gene*. 2013;525(2):162–169. doi: 10.1016/j.gene.2013.03.137
3. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. *Sig Transduct Target Ther*. 2021;6:53. doi: 10.1038/s41392-021-00487-6
4. Bashor CJ, Hilton IB, Bandukwala H, Smith DM, Veiseh O. Engineering the next generation of cell-based therapeutics. *Nat Rev Drug Discov*. 2022;21:655–675. doi: 10.1038/s41573-022-00476-6
5. Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. *Sig Transduct Target Ther.* 2020;5:1. doi: 10.1038/s41392-019-0089-y
6. Uddin F, Rudin CM, Sen T. CRISPR gene therapy: applications, limitations, and implications for the future. *Front Oncol*. 2020;10:1387. doi: 10.3389/fonc.2020.01387
7. Lundstrom K. Viral vectors in gene therapy. *Diseases*. 2018;6(2):42. DOI: 10.3390/diseases6020042
8. Baker M. Digital PCR hits its stride. *Nat Methods*. 2012;9(6):541–544. doi: 10.1038/nmeth.2027
9. Lou Y, Chen C, Long X, et al. Detection and quantification of chimeric antigen receptor transgene copy number by droplet digital PCR versus real-time PCR. *J Mol Diagn.* 2020;22(5):699–707. doi: 10.1016/j.jmoldx.2020.02.007
10. Lin H-T, Okumura T, Yatsuda Y et al. Application of droplet digital PCR for estimating vector copy number states in stem cell gene therapy. *Hum Gene Ther Methods*. 2016;27(5):197–208. doi: 10.1089/hgtb.2016.059
11. Dobnik D, Kogovšek P, Jakomin T, et al. Accurate quantification and characterization of adeno-associated viral vectors. *Front Microbiol*. 2019;10:1570. doi:10.3389/fmicb.2019.01570
12. Furuta-Hanawa B, Yamaguchi T, Uchida E. Two-dimensional droplet digital PCR as a tool for titration and integrity evaluation of recombinant adeno-associated viral vectors. *Hum Gene Ther Met*. 2019;30(4):127–136. doi: 10.1089/hgtb.2019.031
13. Murphy LA, Marians RC, Miller K, et al. Digital polymerase chain reaction strategies for accurate and precise detection of vector copy number in chimeric antigen receptor T-cell products. *Cytotherapy*. 2023;25(1):94–102. doi: 10.1016/j.jcyt.2022.09.004
14. Fehse B, Badbaran A, Berger C, et al. Digital PCR assays for precise quantification of CD19-CAR-T cells after treatment with axicabtagene ciloleucel. *Mol Ther Methods Clin Dev*. 2020;16:172–178. doi: 10.1016/j.omtm.2019.12.018
15. Moutsatsou P, Ochs J, Schmitt RH, Hewitt CJ, Hanga MP. Automation in cell and gene therapy manufacturing: from past to future. *Biotechnol Lett*. 2019;41:1245–1253. doi: 10.1007/s10529-019-02732-z
16. Huggett JF, Foy CA, Benes V, et al. The digital MIQE guidelines: Minimum information for publication of quantitative digital PCR experiments. *Clin Chem*. 2013;59(6):892–902. doi: 10.1373/clinchem.2013.206375

© 2025 Bio-Rad Laboratories, Inc.

How ctDNA Analysis Takes Cancer From Bloodstreams to Breakthroughs

Introduction

When healthy cells break down, their DNA is released into the bloodstream. This is termed cell-free DNA (cfDNA). In patients with cancer, the concentration of cfDNA in blood samples increases significantly and it does so in line with the progression stage of the cancer. Between 0.01% and 90% of the cfDNA in cancer patients originates from tumors. The increase in cfDNA is due to the additional deposition of DNA in the bloodstream released by malignant tumors, called circulating tumor DNA (ctDNA).

ctDNA has emerged as one of the most informative biomarkers in modern oncology research, and it can be detected through liquid biopsies, such as a standard blood test. This provides a non-invasive, repeatable and convenient way to detect cancer and monitor disease progression. Its adoption has opened new opportunities for cancer researchers to study dynamic tumor biology without the limitations of repeated tissue biopsies. This article explores approaches to circulating tumor DNA (ctDNA) analysis, recognizing the value of broad genomic profiling in early research while emphasizing the need for highly sensitive, quantitative tools for ongoing monitoring and clinical investigation.

ctDNA: A real-time molecular window into cancer

The non-invasive nature of ctDNA sampling goes hand in hand with one of its most clinically relevant characteristics – its short half-life, typically less than two hours. This provides an opportunity to monitor changes in tumor behavior in near real time. The ease of sample collection also allows researchers to collect serial samples throughout treatment, offering a dynamic picture of tumor response to treatment and the detection of molecular residual disease, a key indicator of relapse.

In contrast to traditional tissue biopsies, which are invasive and offer only a static snapshot of the tumor, ctDNA analysis provides a safer and more scalable method for capturing tumor heterogeneity and tracking disease progression across multiple time points. For instance, a plasma analysis study identified common driver mutations in *EGFR* and *KRAS* in a matter of days, potentially enabling the detection of *EGFR* T790M drug-resistant variants that are otherwise missed in tissue biopsy samples due to high tumor heterogeneity.[1]

However, ctDNA is typically present in extremely low concentrations, especially in early-stage disease or after treatment. Detecting and quantifying these rare fragments requires high-performance molecular tools capable of exceptional sensitivity, reproducibility and speed. While various methods are suitable, digital PCR (dPCR) has become a preferred method for addressing these demands, enabling researchers to harness the full potential of ctDNA in longitudinal cancer studies and biomarker-driven research.

Choosing the right method for ctDNA detection

Next-generation sequencing (NGS) has the power to rapidly sequence large quantities of DNA, making it a contender

© 2025 Bio-Rad Laboratories, Inc.

for analyzing ctDNA concentration in blood samples. By providing a comprehensive view of the genetic composition of tumors, it can identify known and unknown mutations, including copy number variations and structural rearrangements. For example, a study found that NGS could identify actionable mutations from non-small cell lung cancer (NSCLC) plasma samples.[2] This method enabled more effective personalized therapy in a higher percentage of patients compared to when mutations were identified using NGS on tissue samples.

However, NGS is not without limitations. The cost, time requirements and need for specialized expertise make NGS less practical and accessible than alternative approaches like dPCR. Nevertheless, NGS remains essential in the early stages of clinical research, particularly in the diagnostic stage, due to its unparalleled breadth of screening. It is especially relevant in situations where specific mutations have not yet been identified or when a broad survey of the genome is needed.

On the other hand, dPCR, specifically Droplet Digital PCR (ddPCR), addresses the limitations of NGS and consequently has emerged as a leading method in ctDNA analysis. This technique is rapid, precise and highly sensitive, ideal for detecting rare genetic variants in ctDNA, which may comprise only 0.01% of cfDNA in a sample. The qualities of this technique make it highly suitable for a range of applications in cancer analysis (Figure 1).

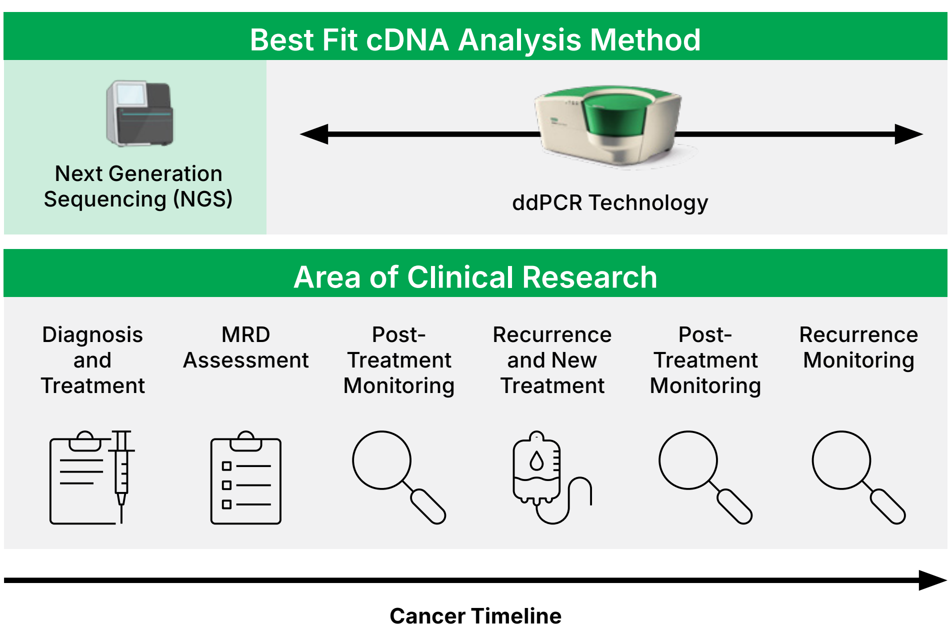


Figure 1. NGS remains essential in the diagnosis and treatment stage of clinical research, while ddPCR technology is most effective in subsequent analysis.

Applications of digital PCR in ctDNA research

A broad portfolio of analytically validated ddPCR assays has been established to target high-value cancer mutations, supporting researchers in the investigation of clinically relevant biomarkers. The effectiveness of this approach has been demonstrated across multiple cancer types in clinical research studies.

Breast cancer

Aside from initial diagnosis and subsequent treatment, a vital part of disease management is detecting and addressing relapses. Molecular residual disease (MRD) refers to the small number of cancer cells remaining in the body after treatment. Although they are strong indicators of relapses in many cancers, standard screening methods often fail to detect them. ddPCR technology has been shown to accurately predict relapse in early-stage breast cancer, while ctDNA sequencing has been used to identify MRD-associated mutations more effectively than sequencing the primary tumor.[3] Research has also found that after treatment for triple-negative breast cancer, the absence of ctDNA in ddPCR analysis was linked to a reduced risk of relapse and better overall prognosis.[4]

Non-small cell lung cancer

Standard clinical monitoring of NSCLC involves imaging methods that identify gross changes in tumor size or structure. However, single-gene ddPCR analysis has demonstrated its value as a cost-effective tool capable of predicting early disease progression and sustained responses to immune checkpoint inhibitors during treatment.[5]

Melanoma

ddPCR technology has also been applied to monitoring treatment response in patients with melanoma. While traditionally monitored by computer tomography, this imaging technique is expensive and time-consuming. Studies exploring how ddPCR solutions can help to predict treatment response by measuring ctDNA in melanomas have revealed that ddPCR technology achieves higher sensitivity than NGS and does so at a faster pace and lower cost.[6] The technique has also been used to predict survival and treatment outcomes of melanoma patients.[7]

Colorectal cancer

Using current imaging and laboratory methods, it is notoriously challenging to detect relapses in bowel cancer patients. However, through ddPCR-

© 2025 Bio-Rad Laboratories, Inc.

based ctDNA analysis, early changes in ctDNA levels could signal disease progression, facilitating prompt clinical response and better outcomes.[8]

Advancing research through longitudinal monitoring

One of the most impactful advantages of ddPCR technology is its ability to support frequent, minimally invasive ctDNA sampling throughout treatment courses. This longitudinal approach provides insights that static tissue biopsies cannot – revealing changes in tumor dynamics, emerging resistance mechanisms and evolving biomarker profiles across multiple cancer types.

As a result, ddPCR technology is not only accelerating biomarker discovery but also enabling more adaptive and responsive clinical study designs. In some research settings, ctDNA levels measured by ddPCR solutions are being evaluated as surrogate endpoints in clinical trials. These molecular endpoints may allow for earlier evaluation of therapeutic efficacy, potentially reducing trial duration, cost and participant burden.

ctDNA and ddPCR technology: A partnership for personalized medicine

As precision oncology advances, the partnership between ctDNA analysis and dPCR is proving essential to both translational cancer research and the development of truly personalized cancer care. ctDNA provides a dynamic, minimally invasive view of tumor genetics, while dPCR delivers the sensitivity, precision and quantification needed to track those changes with confidence. Together, they enable researchers to detect rare variants, monitor treatment response and assess MRD across a range of cancer types and study designs. This powerful combination is helping translate real-time molecular insights into more informed clinical decisions, bringing personalized medicine closer to routine practice.

Conclusion

The use of Droplet Digital PCR for ctDNA analysis is increasingly being recognized as the cornerstone of modern liquid biopsy research and central to cancer research. While broad genomic profiling through NGS remains important in the early stages of cancer research, clinical settings often require a more focused approach – one that can detect rare mutations with high sensitivity, deliver rapid results and integrate seamlessly into everyday workflows.

The accuracy and ease of use of dPCR have made it a trusted method for researchers studying a wide range of cancers and trial designs. As oncology moves toward more adaptive, personalized treatment strategies, the need for real-time monitoring of tumor genetics continues to grow, and dPCR is well-positioned to meet that demand.

Learn more here

References

1. Sacher AG, Paweletz C, Dahlberg SE, et al. Prospective validation of rapid plasma genotyping for the detection of EGFR and KRAS mutations in advanced lung cancer. *JAMA Oncol*. 2016;2(8):1014–1022. doi: 10.1001/jamaoncol.2016.0173
2. Aggarwal C, Thompson JC, Black TA, et al. Clinical implications of plasma-based genotyping with the delivery of personalized therapy in metastatic non–small cell lung cancer. *JAMA Oncol*. 2019;5(2):173–180. doi: 10.1001/jamaoncol.2018.4305
3. Garcia-Murillas I, Schiavon G, Weigelt B, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. *Sci Transl Med*. 2015;7(302):302ra133. doi: 10.1126/scitranslmed.aab0021
4. Cavallone L, Aguilar-Mahecha A, Lafleur J, et al. Prognostic and predictive value of circulating tumor DNA during neoadjuvant chemotherapy for triple negative breast cancer. *Sci Rep*. 2020;10:14704. doi: 10.1038/s41598-020-71236-y
5. van der Leest P, Hiddinga B, Miedema A, et al. Circulating tumor DNA as a biomarker for monitoring early treatment responses of patients with advanced lung adenocarcinoma receiving immune checkpoint inhibitors. *Mol Oncol.* 2021;15(11):2910–2922. doi: 10.1002/1878-0261.13090
6. Forthun RB, Hovland R, Schuster C, et al. ctDNA detected by ddPCR reveals changes in tumour load in metastatic malignant melanoma treated with bevacizumab. *Sci Rep*. 2019;9:17471. doi: 10.1038/s41598-019-53917-5
7. Syeda MM, Wiggins JM, Corless BC, et al. Circulating tumour DNA in patients with advanced melanoma treated with dabrafenib or dabrafenib plus trametinib: a clinical validation study. *Lancet Oncol*. 2021;22(3):370–380. doi: 10.1016/S1470-2045(20)30726-9
8. Holm M, Andersson E, Osterlund E, et al. Detection of KRAS mutations in liquid biopsies from metastatic colorectal cancer patients using droplet digital PCR, Idylla, and next generation sequencing. *PloS One*. 2020;15(11):e0239819. doi: 10.1371/journal.pone.0239819

© 2025 Bio-Rad Laboratories, Inc.

Droplet Digital PCR Revolutionizes Vaccine Development and Manufacturing

Modern vaccine development demands unprecedented precision in measuring and characterizing antigens, immune responses and product quality. Traditional analytical methods often fall short of the sensitivity and precision requirements needed, contributing to development timelines that can span 5–10 years. Analytical bottlenecks throughout the development pipeline create delays that ultimately impact global health outcomes. This is particularly evident during public health emergencies like the COVID-19 pandemic.[1]

Droplet Digital PCR (ddPCR) has emerged as a transformative analytical technology that addresses these challenges across vaccine research and manufacturing. By partitioning reactions across thousands of droplets, ddPCR technology enables absolute quantification without standard curves – making it the gold standard for viral vector genome titer determination.

This article demonstrates how ddPCR technology supports every stage of vaccine development, from initial antigen identification through large-scale manufacturing and quality control. By highlighting specific applications, it explores how this technology is accelerating vaccine development timelines while enhancing product safety and efficacy profiles.

The evolution of vaccine analytics

Vaccine development has evolved dramatically, encompassing diverse platforms, each of which present unique analytical challenges. Modern vaccines include whole-organism vaccines (both live attenuated and inactivated), subunit vaccines containing specific antigenic components, viral vector platforms that deliver genetic material and nucleic acid vaccines including mRNA and DNA constructs.[2,3] Each platform requires specialized analytical approaches for development and quality control, from characterizing complex protein structures in subunit vaccines to ensuring precise genetic payload delivery in viral vector systems.

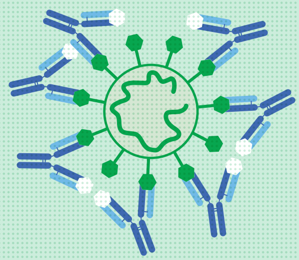
Traditional analytical methods have struggled to meet the demanding requirements of these advanced vaccine platforms. Conventional qPCR methods rely on standard curves for quantification, introducing variability and potential bias that can compromise analytical accuracy. These methods also struggle to detect low-abundance targets or discriminate between closely related sequences. Issues with reproducibility and precision also create regulatory approval bottlenecks, extending development timelines and delaying access to life-saving vaccines.

ddPCR technology represents a step forward through its partition-based approach, which eliminates standard curve dependency by distributing the sample across thousands of individual reaction chambers. ddPCR solutions integrate throughout the complete vaccine development pipeline, from initial antigen discovery and characterization through to large-scale manufacturing and quality control processes (Figure 1).

This approach enables direct counting of target molecules, providing absolute quantification with superior precision and significantly reduced bias. This generates more reliable data

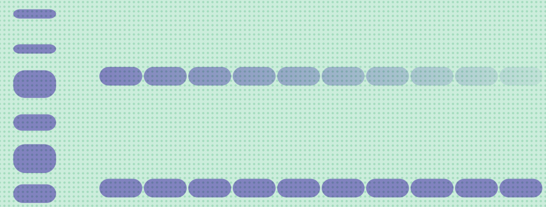
Building a Better Vaccine

Throughout vaccine research and development, scientists must analyze their antigens, track the immune response and determine viral loads. Modern instruments and assays aid vaccine developers and ensure that they receive fast and reliable results.



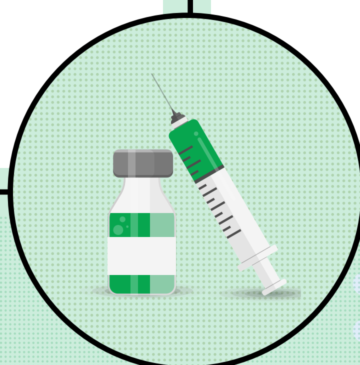
Identify an Antigen

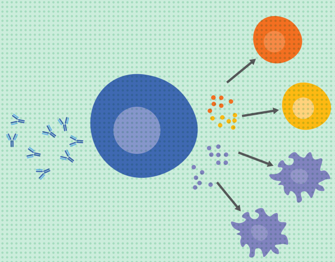
- Find an antigen that induces protective immunity by assessing interactions with neutralizing antibodies using the ZE5 Cell Analyzer.
- Detect antigen variants with Droplet Digital PCR (ddPCR) using a QX200 or QX ONE instrument.



Verify Expression

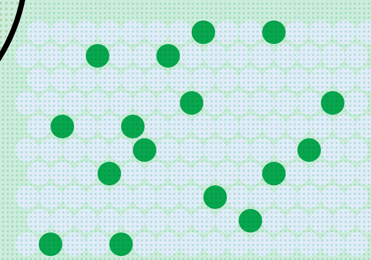
- Develop a vaccine that delivers the candidate antigen or its sequence.
- Assess antigen production and purity in the chosen system by western blot and SDS-PAGE.





Analyze the Immune Response

- Track immune responses to immunization by measuring chemokines and cytokines with Bio-Plex Immunoassays.
- Phenotype immune cells that respond to vaccination with the ZE5 Cell Analyzer.



Verify Immunity

- Determine the viral load required to induce pathogenicity with ddPCR systems.
- Quantitate viral titer for vaccine challenge experiments with ddPCR systems.

Figure 1. ddPCR solutions in the vaccine development pipeline.

throughout the vaccine development pipeline, supporting faster decision-making and more confident regulatory submissions.[4]

ddPCR Technology enables precision throughout vaccine R&D

The analytical precision of Droplet Digital PCR can be applied to multiple stages of vaccine research and development, beginning with antigen identification and characterization. The technology is able to detect single-nucleotide variants and discriminate between closely related viral or bacterial strains. This capability enables researchers to track emerging variants within populations, which proved essential during the COVID-19 pandemic, and it helps them make informed decisions about vaccine formulation, ensuring continued efficacy against evolving pathogens.[5,6]

Cell line development represents another application where ddPCR solutions'precision drive manufacturing success. Production cell lines must express target antigens consistently over numerous passages while maintaining genetic stability. ddPCR technology precisely measures transgene copy numbers, providing researchers with reliable data to assess genetic stability and identify high-expressing, stable clones.[7] Accurate copy number determination is essential for establishing production cell lines that can reliably manufacture vaccine antigens at a commercial scale while meeting stringent quality requirements.

Immune response analysis and vaccine efficacy testing also benefit significantly from ddPCR technology's quantification capabilities. The technology plays a role in viral load quantification for vaccine challenge experiments, where precise measurements are essential for determining protective efficacy. Droplet Digital PCR enables researchers to establish accurate viral dosages required for pathogenicity studies. This ensures that challenge experiments provide meaningful data while maintaining appropriate safety margins. This precision is particularly important in preclinical studies where dosage accuracy directly impacts the validity of efficacy conclusions.

Viral vector vaccine development has embraced ddPCR solutions as the gold standard for genome titer determination.[8] Traditional qPCR methods often struggle with the complex matrices and potential inhibitors present in viral vector preparations, while ddPCR's partition-based approach provides more robust and accurate quantification. The technology measures vector infectivity and payload delivery to host cells, enabling developers to ensure efficacious, non-toxic dosing. This has become increasingly important as viral vector platforms gain prominence for vaccines against infectious diseases and in gene therapy applications.

Quality control and manufacturing excellence

Large-scale vaccine manufacturing demands rigorous quality control measures, and ddPCR technology is an important tool for ensuring product consistency and safety. Impurity detection is a critical quality control application where ddPCR solutions' sensitivity provide significant advantages. The technology detects minute quantities of host cell DNA (HCD) contamination, a regulatory concern that requires careful monitoring throughout manufacturing processes.[9,10] Unlike traditional methods that may require extensive sample preparation, ddPCR method provides direct quantification without DNA purification steps, streamlining quality control workflows while maintaining regulatory compliance.

Regulatory agencies worldwide have established strict requirements for HCD reporting in biological products, and ddPCR has become the preferred method for meeting these standards.[9] The technology reliably detects HCD levels below regulatory thresholds, providing manufacturers with confidence in their product purity assessments. Additionally, ddPCR solutions provide DNA size measurements that help characterize the nature of any detected contamination, supporting comprehensive safety evaluations.

Potency testing for advanced vaccine platforms presents unique challenges that ddPCR method addresses effectively. Nucleic acid vaccines, including mRNA constructs encased in lipid nanoparticles, require precise measurement of active ingredient concentrations to ensure consistent dosing. Droplet Digital PCR accurately quantifies these complex formulations, determining both the concentration of delivery vehicles and the integrity of genetic payloads.[11] For viral vector vaccines, the technology measures payload delivery within host cells, ensuring that each vaccine dose contains the optimal amount of active genetic material.

Manufacturing scalability and consistency depend on robust analytical methods that can maintain accuracy across different production scales and facilities. ddPCR solutions ensure batch-to-batch consistency in large-scale production by providing reliable, standardized measurements that are independent of operator variability or standard curve preparation. The technology supports real-time quality monitoring throughout manufacturing processes, enabling rapid detection of any deviations that could impact product quality. This is particularly valuable for meeting stringent regulatory requirements for vaccine approval, where consistent analytical data across multiple manufacturing sites may be required.

© 2025 Bio-Rad Laboratories, Inc.

Conclusions and future prospects

ddPCR technology has already transformed vaccine development and manufacturing practices by providing the analytical precision and reliability required for modern vaccine platforms. The technology enables faster, more precise vaccine development timelines by eliminating analytical bottlenecks that historically contributed to lengthy development cycles.

The major advantages of ddPCR solutions across the vaccine development pipeline include absolute quantification without standard curves, superior precision and reduced bias, enhanced sensitivity for detecting low-abundance targets and robust performance in complex sample matrices. These capabilities make ddPCR technology an essential tool for antigen characterization, cell line development, efficacy testing and manufacturing quality control. As vaccine platforms continue to evolve and become more sophisticated, the analytical demands will only increase, making Droplet Digital PCR adoption increasingly critical for success.

Discover how ddPCR solutions can accelerate your vaccine development programs

References

1. Chaudhary N, Weissman D, Whitehead KA. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. *Nat Rev Drug Discov*. 2021;20(11):817–838. doi: 10.1038/s41573-021-00283-5
2. Liu MA. A comparison of plasmid DNA and mRNA as vaccine technologies. *Vaccines*. 2019;7(2):37. doi: 10.3390/vaccines7020037
3. Ura T, Okuda K, Shimada M. Developments in Viral Vector-Based Vaccines. *Vaccines*. 2014;2(3):624–641. doi: 10.3390/vaccines2030624
4. Vasudevan HN, Xu P, Servellita V, et al. Digital droplet PCR accurately quantifies SARS-CoV-2 viral load from crude lysate without nucleic acid purification. *Sci Rep*. 2021;11:780. doi: 10.1038/s41598-020-80715-1
5. Ciesielski M, Blackwood D, Clerkin T, et al. Assessing sensitivity and reproducibility of RT-ddPCR and RT-qPCR for the quantification of SARS-CoV-2 in wastewater. *J Virol Methods*. 2021;297:114230. doi: 10.1016/j.jviromet.2021.114230
6. Liu H, Wu NC, Yuan M, et al. Cross-neutralization of a SARS-CoV-2 antibody to a functionally conserved site is mediated by avidity. *Immunity*. 2020;53(6):1272–1280.e5. doi: 10.1016/j.immuni.2020.10.023
7. Berman J, Heredia N, Regan J et al. Droplet digital PCR: High-resolution copy number variation analysis; Bulletin 6475. Bio-Rad. www.bio-rad.com/sites/default/files/webroot/web/pdf/lsr/literature/Bulletin_6475.pdf. Accessed August 5, 2025.
8. Alliance for Regenerative Medicine, National Institute for Innovation in Manufacturing Biopharmaceuticals. Generation of a quality target product profile. In: *Project A-Gene*. 2021. https://alliancerm.org/wp-content/uploads/2021/09/ALL-PROJECT-A-GENE-V10.pdf. Accessed August 5, 2025.
9. World Health Organization. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks, Annex 3, TRS No 978. https://www.who.int/publications/m/item/animal-cell-culture-trs-no-978-annex3. Published May 22, 2013. Accessed August 5, 2025.
10. Do D, Ma M, Klitgord N, Regan J, Ngo N. Direct quantification of residual host cell DNA. Bio-Rad. www.bioradiations.com/wp-content/uploads/2015/05/direct-quantification-of-residual-host-cell-dna.pdf. Accessed August 5, 2025.
11. Mucker EM, Karmali PP, Vega J, et al. Lipid nanoparticle formulation increases efficiency of DNA-vectored vaccines/immunoprophylaxis in animals including Transchromosomic Bovines. *Sci Rep*. 2020;10:8764. doi: 10.1038/s41598-020-65059-0

© 2025 Bio-Rad Laboratories, Inc.

Resources

Become a Master of ddPCR

Digital PCR is transforming the modern molecular biology lab and it's easier to master than you might think!

With its simple technology and chemistry, the key to success lies in understanding how it works in real-world conditions.

This video series will guide you through both foundational concepts and advanced applications, helping you build the expertise needed to master digital PCR.

Watch the videos here to unlock the full potential of digital PCR!

© 2025 Bio-Rad Laboratories, Inc.