tetrascience

ddPCR in gene therapy: Solving data workflow challenges

SOLUTION BRIEF

Gene therapies are quickly moving from hype to reality. The U.S. Food and Drug Administration (FDA) recently approved two gene therapies for sickle cell disease.[1] Clinical trials of another gene therapy restored hearing in congenitally deaf children.[2] By 2025, the FDA expects to greenlight ten to twenty cell and gene therapy products annually.[3]

Gene therapies treat diseases by replacing a faulty gene with a healthy copy, deactivating a malfunctioning gene, or introducing a new or modified gene to combat the illness. A vector, such as the harmless adeno-associated virus, delivers genetic material to target cells.

Scientists developing and manufacturing gene therapies need to be able to measure the quantity and quality of the vector as well as evaluate cells treated with the therapy. Droplet digital polymerase chain reaction (ddPCR) is increasingly being used for these purposes. Here, we discuss the applications of ddPCR in gene therapy and the data challenges faced by scientists who use the technique. We'll also explore how the Tetra Scientific Data and AI Cloud™ improves ddPCR workflows for better scientific outcomes.

Benefits of ddPCR

Droplet digital PCR is a highly precise and sensitive method for quantifying specific nucleic acid sequences. Its progenitor, standard PCR, has been widely used for decades but is not inherently quantitative. It amplifies DNA exponentially until the raw materials are exhausted, making it impossible to use the final product amount as a reliable indicator for the initial number of target DNA molecules.

Quantitative PCR (qPCR) attempts to address this limitation by measuring the amplification of DNA in real time using fluorescent probes or dyes. However, qPCR has several drawbacks, including (1) the need for calibration curves that add variability and require qualified reference material, (2) dilution bias, which impacts the lower limit of detection, and (3) a dynamic range that depends on the efficiency of PCR amplification. In contrast, ddPCR offers better precision, sensitivity, and reliability—all without requiring a calibration curve.[4] These advantages have made it a popular method for quantifying nucleic acid sequences, especially in the development and quality control of gene therapies.

ddPCR use cases in gene therapy

Droplet digital PCR can analyze gene therapy vectors (prior to administration) and cells treated with those therapies (post-administration). Here are several common use cases for ddPCR in gene therapy.

- **Viral titration:** Measuring viral titer is important at all stages of gene therapy development and manufacturing, much like assessing the concentration of a traditional drug. ddPCR can directly quantify copies of the packaged viral genome.
- **Viral genome integrity:** Not all viral particles produced for gene therapy contain complete and correct genetic material. By probing multiple parts of the vector genome with ddPCR, scientists can measure the number of viral particles with a fully intact genome sequence.
- **Integration site analysis:** ddPCR helps identify where therapeutic genes integrate into the genome of the target cells, which is vital for assessing the safety and efficacy of gene therapies.
- **Copy number variation:** ddPCR can also measure how many copies of the engineered gene were introduced into the target cells.
- **Monitoring gene editing:** When the genetic payload contains gene editing technologies like CRISPR, ddPCR provides a reliable method to quantify on-target and off-target effects.
- **Contaminant detection:** Contaminants, including residual DNA from host cells, plasmids, or helper viruses, pose significant safety risks and can affect the efficacy of gene therapy. ddPCR can detect contaminant DNA sequences with high specificity and sensitivity.

How ddPCR works—the basics

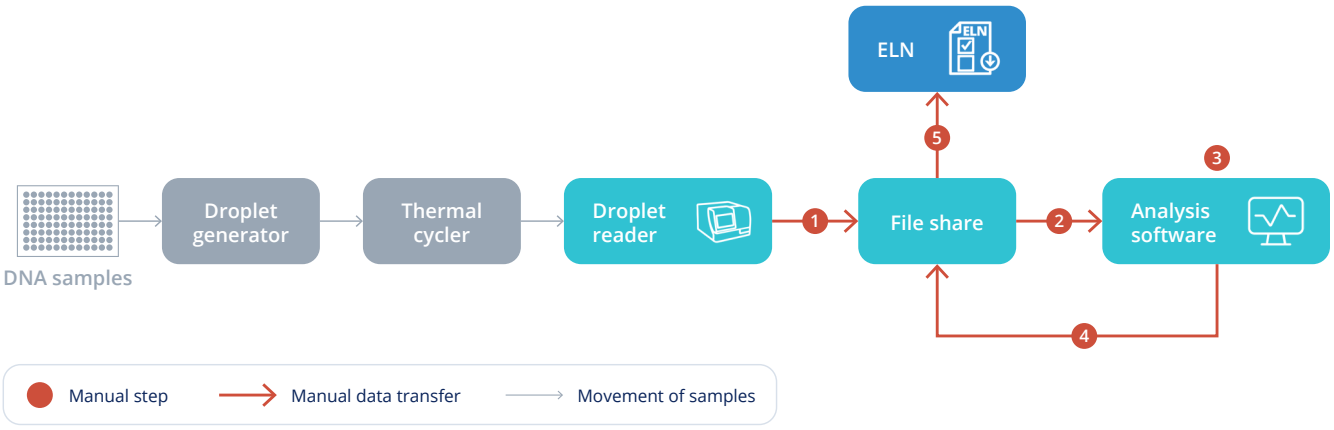
To better understand the data workflow for ddPCR, let's first take a brief look at how the technology works.

1. **Sample preparation:** The process begins with the preparation of a sample mix containing the target nucleic acids, primers, probes, and a master mix for PCR.
2. **Droplet generation:** This mix is then partitioned into thousands of tiny, uniform droplets using a droplet generator. Each water-in-oil droplet acts as an individual microreactor, encapsulating a single or few nucleic acid molecules.
3. **PCR amplification:** The droplets are subjected to temperature cycling which triggers the amplification reaction. During this process, the probes bind to the target sequence, if present, and release a fluorescent dye that can be detected in the next step. Probes of different colors can be multiplexed in the reaction to simultaneously detect more than one DNA sequence.
4. **Detection:** After amplification, the droplets pass through a droplet reader, which uses fluorescence to count the number of "positive" droplets (containing the amplified target sequence) and "negative" droplets (without the target). Since each droplet is an independent reaction space, the count of fluorescent droplets directly reflects the number of target DNA molecules present in the original sample. The technique is called "digital" because the ultimate readout of each droplet is binary—positive or negative—for each target DNA sequence.
5. **Quantification:** By applying Poisson statistics to the ratio of positive to total droplets, ddPCR precisely quantifies the absolute concentration of the target nucleic acid in the original sample.

Data challenges of ddPCR

Scientists performing ddPCR often have to manually transcribe and transfer data multiple times per assay (see figure below). Consider a typical workflow using the Bio-Rad QX200 ddPCR system, which consists of two devices: a droplet generator and a droplet reader. After droplet generation and PCR amplification, samples are run on the droplet reader. Raw data (a QLP file) is manually retrieved from the instrument's computer, uploaded to a storage drive, and then transferred to a second computer containing QuantaSoft analysis software. There, the scientist calculates the nucleic acid concentration per target sequence for each sample. Results are manually exported in a CSV file, archived in the storage drive, and finally transcribed into the electronic lab notebook (ELN).

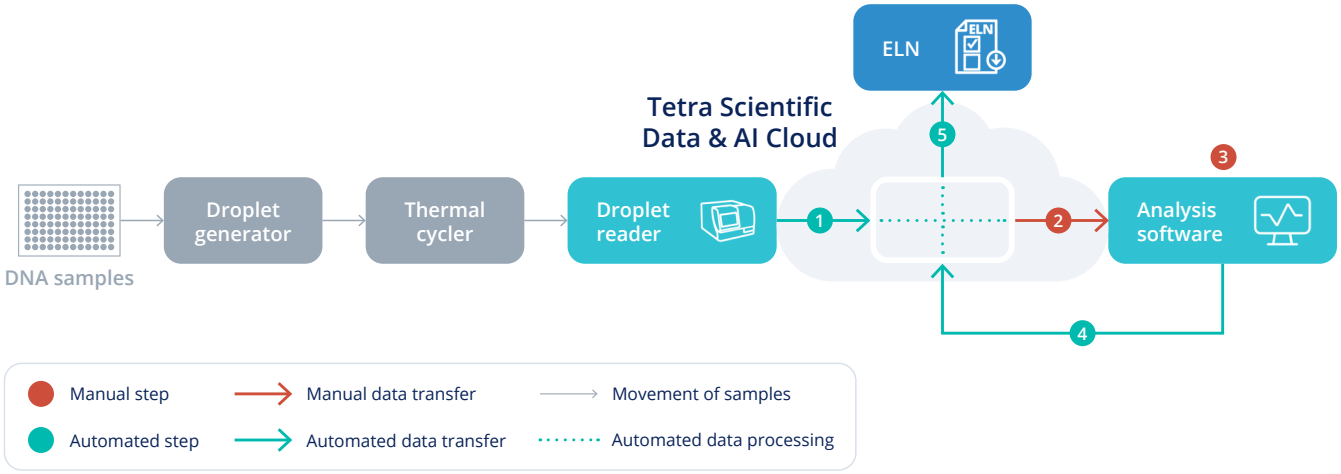
Given its reliance on manual steps, the entire process is time consuming and prone to errors. Plus, scientists may struggle to find data from previous experiments when assembling larger datasets or performing additional analyses.



A common workflow for ddPCR. (1) Raw data from the droplet reader (e.g., QLP file) is uploaded to a file share. (2) The data is then imported into the analysis software (e.g., QuantaSoft) on the scientist's personal computer. (3) The scientist performs the analysis to compute the nucleic acid concentration. (4) The results are exported in a CSV file and uploaded to the file share. (5) The scientist transcribes the results into the ELN.

Streamline and enhance the ddPCR workflow

The Tetra Scientific Data and AI Cloud accelerates and improves the ddPCR workflow through data replatforming and engineering. As illustrated below, the platform eliminates many of the manual steps through automation. The upshot is fewer errors and faster assays. Results are centralized in the cloud and automatically contextualized with metadata such as sample ID, user name, and date. This makes searching, aggregating, and monitoring data far easier.



A ddPCR workflow with the Tetra Scientific Data and AI Cloud. (1) Raw data from the droplet reader (e.g., QLP file) is automatically ingested into the Tetra Scientific Data and AI Cloud. (2) The data is then imported into the analysis software (e.g., QuantaSoft) on the scientist's personal computer. (3) The scientist performs the analysis to compute the nucleic acid concentration. (4) The platform automatically ingests the CSV results and engineers them into Tetra Data. (5) The results are automatically pushed to the ELN.

The Tetra-enabled workflow also transforms the ddPCR results into analytics- and AI-ready Tetra Data. The data is harmonized into an open, vendor-agnostic format with scientifically relevant taxonomies and ontologies.

With Tetra Data, gene therapy groups can easily access compliant, liquid data to rapidly assemble large-scale datasets for analytics and AI. For instance, ddPCR results can be integrated with data from other sources—such as next-generation sequencing, microarray, and mass spectroscopy—to build powerful data models. These models may predict vector titer, optimize formulation, or forecast transduction efficiency. The resulting insights could be profound: safer and more effective gene therapies, reduced costs, and faster time to market.

Next steps

Ready to transform your ddPCR workflow? Reach out to one of our experts to discuss your use case.

References

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[3] Scott Gottlieb, "Statement from FDA Commissioner Scott Gottlieb, M.D. and Peter Marks, M.D., Ph.D., Director of the Center for Biologics Evaluation and Research on new policies to advance development of safe and effective cell and gene therapies," U.S. Food and Drug Administration, January 15, 2019, https://www.fda.gov/news-events/press-announcements/statement-fda-commissioner-scott-gottlieb-md-and-peter-marks-md-phd-director-center-biologics.

[4] Yun Zhao, Qingyan Xia, Youping Yin, and Zhongkang Wang, "Comparison of Droplet Digital PCR and Quantitative PCR Assays for Quantitative Detection of Xanthomonas citri Subsp. citri," *PLOS One* 11, no. 7 (2016), https://doi.org/10.1371/journal.pone.0159004.