The 6-color naica® system: a digital PCR platform and workflow for high multiplex genetic detection and analysis

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Abstract

There are several molecular platforms routinely used to investigate genomic material. Quantitative polymerase chain reaction (qPCR) is ubiquitously used because of the ease of use and the precision however, it does not offer high multiplexing. While digital PCR (dPCR) is known for increased precision and sensitivity, most digital PCR platforms provide the same level of multiplexing as qPCR. Next-generation sequencing (NGS) is less precise than qPCR or dPCR and but has the highest multiplexing capability of the three technologies. Conventional digital PCR systems have been limited by the number of instruments required to conduct the workflow, time-to-result compared to qPCR, and level of multiplexing as compared to next-generation sequencing (NGS). Crystal Digital PCR™ and the naica system is a next-generation digital PCR platform that simplifies the number of instruments in the workflow, speeds up the time of results to less than 3 hours, and increases the number of detection colors to 6 spectrally distinct channels. By using a combination of dual-labelled fluorescent probes, multiplexed digital PCR provides a simple solution that makes it possible to use less of a precious sample when faced with limited quantity without sacrificing sensitivity. The naica system workflow utilizes microfluidic consumables, such as the Sapphire chip for high sensitivity applications, and the Opal chip for higher throughput studies. All reagents including primers and probes, master mix, and sample are inputted into the chips in one single loading step. Chips are transferred to the Geode instrument, where the sample is partitioned into a 2D-monolayer of droplet crystals and where all targets are amplified. After the PCR program has been completed, the same chips are then loaded into the Prism6 imager which detects all targets across 6 fluorescent channels and outputs an image of all droplets in a sample. Finally, Crystal Miner analysis software can be used to analyze and decipher the quantifications and expression of all targets in the assay. The 6-color naica system allows for high multiplex detection of targets previously unattainable by conventional digital PCR systems. With this advent, genomic scientists have an additional platform in the molecular toolbox that supports seamless assay development of oncology biomarker assays for gene expression, rare target detection, and liquid biopsy applications.

The Next Generation of PCR

dPCR is the newest evolution of PCR, which builds on some of the many advantages of qPCR, including its high precision, while providing some new advantages. dPCR provides an absolute quantification of target nucleic acids without the need of a standard curve (**Figure 1**). By separating the bulk reaction mixture into smaller droplets, dPCR provides increased sensitivity along with a unique ability to quantify low concentration targets at the same time as high concentration targets. This is a principle that qPCR struggled with due to reagent competition between varying concentrations of targets. Following amplification of the droplets containing the reaction mixture, the law of Poisson can be used to calculate the concentration of a target of interest, using the ratio of positive droplets to total analyzeable droplets (**Figure 2**).

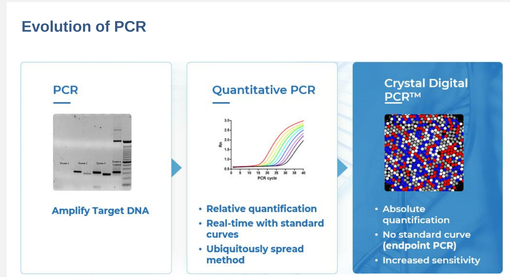


Figure 1. cdPCR is a form of digital PCR that builds upon the advantages of qPCR without the need of a standard curve. In addition, digital PCR allows for increased sensitivity, making it a great method for use with low concentration targets.

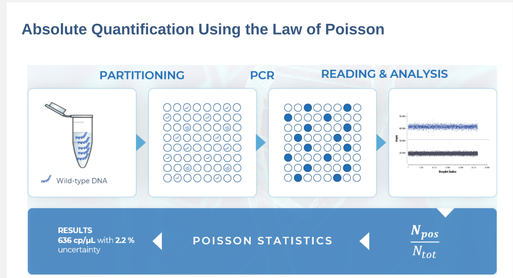


Figure 2. The absolute quantification in cdPCR relies on the Law of Poisson, a statistical method that uses the ratio of positive droplets to total droplets to provide a concentration with its associated 95% convidence interval. A threshold is applied to data automatically to define which droplets are positive and which are negative.

Efficiently Produce Data with a Simplified Workflow

The naica® system provides a simple and user friendly option to perform Crystal Digital PCR™ and get the most data out of the least amount of sample. The workflow of the naica® system consists of 4 main stages: preparation, amplification, reading, and data analysis (**Figure 3**). During the preparation step, Stilla Technologies offers two chips for loaing: the higher sensitivity Sapphire chip and the higher throughput Opal chip (**Figure 4**). During the amplification stage, the Geode performs both a contactless pressure based fluid injection as well as a standard PCR thermocycling program (**Figure 5**). Finally, the Prism 6 is used to scan the chips in six different colors: blue, teal, green, yellow, red, and infrared (**Figure 6**). The naica® system is unique in the fact that it allows any fluorophore that fits within the excitation and emission wavelength ranges for each of these channels, allowing for a multitude of assay design options.

naica® System Overview

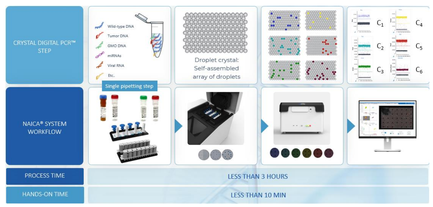


Figure 3. The naica® workflow feature four phases using a total of two instruments. Once the run has been processed in the first three steps, the Crystal Miner software can be used to analyze data.

Chip Based Chemistry

Figure 4. The naica® system features two compatible consumables, the Sapphire and Opal chips. The Sapphire chip is a higher sensitivity option, with a lower LoD, while the Opal chip feature improved throughput.

Pressure Driven Droplet Generation

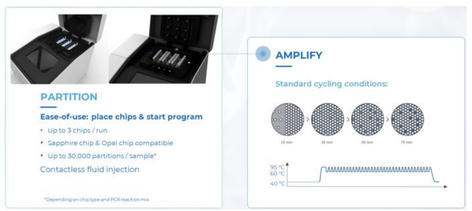
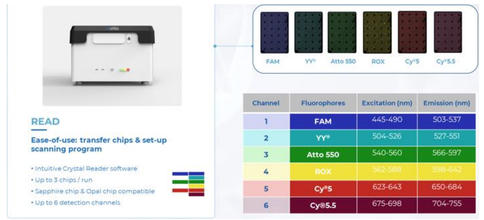


Figure 5. The Geode is a dual function droplet generator and thermocycler. It uses a contactless fluid injection to form the droplets using microfluidic tunnels. The Geode can be custom programmed to use any thermocycling conditions.

Customization in 6 Channels



Channel	Fluorophores	Excitation (nm)	Emission (nm)
1	FAM	445-490	503-537
2	YY¹	504-526	527-551
3	Atto 550	540-560	566-597
4	ROX	582-588	598-642
5	Cy®5	623-643	650-684
6	Cy®5.5	675-698	704-755

Figure 6. The Prism 6 features 6 color detection. The naica® system is compatible with any fluorophores that fit the excitation and emission wavelengths, shown here. Following scanning on the Prism 6, the data is analyzed using Crystal Miner.

Troubleshooting Made Easy with Crystal Miner

The data analysis stage of the naica® system is simplified with the Crystal Miner software. This data analysis software allows you to visualize exactly how the droplets were formed in each chamber of your experiment. This is an incredibly useful tool to troubleshoot any issues with assay design and sample prep. Along with seeing a picture of the droplet formation, Crystal Miner allows your to dive deeper into your data and visualize where positive droplets for each channel lay within the chamber (**Figure 7**). Along with all of the visual data that the Crystal Miner provides, the software allows you to explore the quantification of your data, including the uncertainty graphs associated with the confidence intervals calculated by the Law of Poisson (**Figure 8**)**.** This is a powerful tool that allows you to explore the dynamic range of each type of chip and expand on your understanding of dPCR.

Data Visualization of Droplets

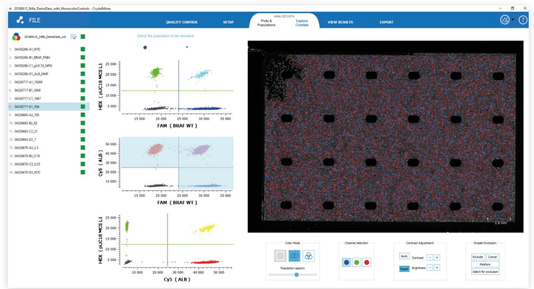


Figure 7. The Explore Crystal tab allows users to expand on their data analysis in a unique way. Images of the droplet formation are shown for each chamber and users have the capability to illuminate droplets with any pofile of positive and negative values for each target of interest.

Uncertainty Curves and Dynamic Range

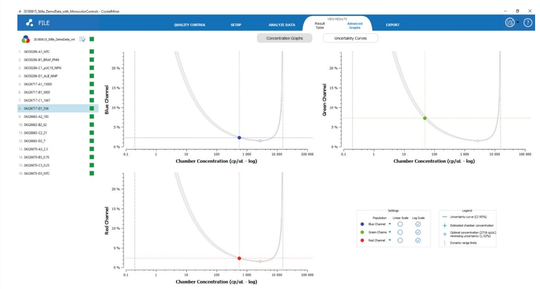


Figure 8. The Uncertainty Graphs tab allows users to explore the relationship between concentration and uncertainty. The Crystal Miner software shows the position of each sample tested along this curve. The software also provides users with a concentration that minimizes their uncertainty.

Additional Information

For more information about 6-color Crystal Digital PCR™ and other naica® products, visit Stilla Technologies' at https://www.stillatechnologies.com/ and posters 75, 527, 534, 2930, 2932, and 2942

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