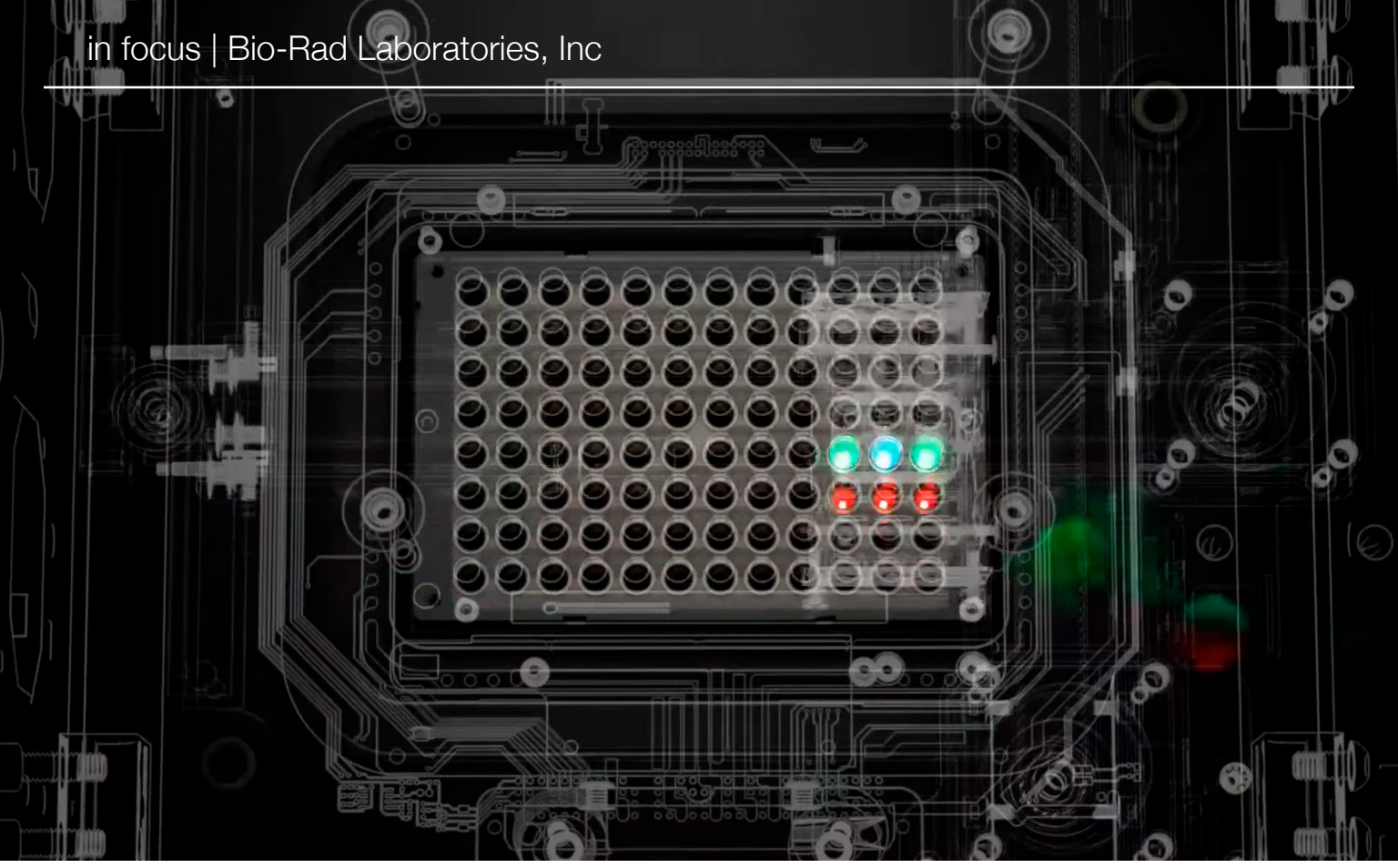
Shuttle-based optical design advances qPCR-based research

Enabling advances in translational research and testing of biopharmaceutical products.

PCR is a simple but indispensable technique used in molecular biology and genetics. First invented in 1983 by American biochemist Kary Mullis, PCR allows scientists to amplify and detect DNA and RNA sequences. A highly sensitive technique, PCR provides a quick means for nucleic acid cloning, taking only a few hours, with minimal templates for detection and amplification of sequences. Its ability to generate large amounts of DNA from a small quantity of nucleic acid lends to its description as a molecular photocopying technique.

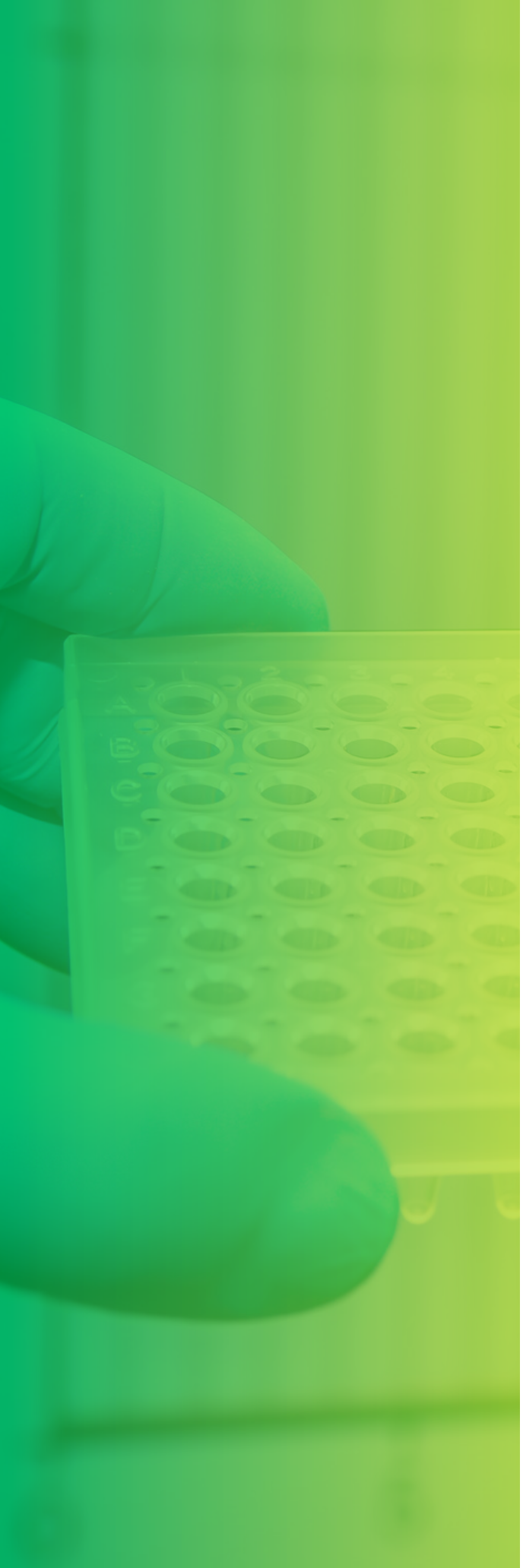
Quantitative PCR (qPCR) expands the conventional PCR process flow and provides improved sensitivity and specificity for the detection and quantification of DNA sequences. Unlike conventional endpoint PCR, qPCR follows the DNA amplification process as the reaction progresses in real-time with resultant product quantification after each cycle. This is achieved by tracking the fluorescence of an intercalating dye or sequence-specific probe using qPCR instrumentation and proprietary software. As the measured fluorescence is proportional to the total amount of product DNA, the change in fluorescence during the reaction's progress can be used to calculate the precise amount of product DNA produced in each cycle. Thus, qPCR can quantify the initial number of copies of the template DNA with high accuracy, sensitivity, and over a wide dynamic range. While conventional



endpoint PCR is considered qualitative or semi-quantitative, qPCR can provide either qualitative or fully quantitative results.

qPCR INSTRUMENTATION

Thermal cyclers or conventional PCR instruments are crucial tools in molecular applications. Consisting of a thermal block with wells that allow for the insertion of PCR reaction mixture tubes, thermal cyclers raise and lower the temperature of the block in discrete timesteps. This facilitates the cyclical temperature processes required for the initialization, denaturation, annealing, and elongation of DNA sequences in the PCR process. A standard qPCR machine also consists of a heated block facilitating cyclical temperature transitions of samples but additionally incorporates a fluorescent source and fluorometer that are utilized to excite fluorophores and detect the output fluorescence during qPCR amplification. qPCR systems use a wide variety of optics combining light sources, filters, and detectors to measure the sample fluorescence in real-time reactions. This may often involve the simultaneous detection of multiple targets in a single reaction well, using different pairs of primers for each target, with various flurophores or channels of colors available for use as probes. This is also known as multiplexing and is now a standard in the development of PCR systems and comparative analyses for many areas of research and testing. Common light sources for sample illumination include light-emitting diodes, halogen lamps, and lasers. Lamp-based optical designs result in varying light paths for illumination and detection for each well in the thermal cycler block. These variations result in differing absolute fluorescence measurements for wells with the same fluorophore concentration. However, these variations do not affect qPCR results as an internal reference dye can be used to normalize well-to-well fluorescence signal differences via qPCR software. Alternatively, a ROX fluorescent dye can also be used as a passive reference detected in a separate channel compared to the reporter dye. Unfortunately, these steps often result in a need to sacrifice data collection in one of the channels.



NOVEL OPTICAL SHUTTLE DESIGN FOR qPCR

The Bio-Rad™ approach uses a novel optical design with a fundamentally different approach toward qPCR. Bio-Rad's CFX Opus Real-Time PCR Systems utilize solid-state light-emitting diodes (LEDs) for fluorescence excitation and photodiodes for detection. Available in three different block configurations including the CFX Opus 96 Real-Time PCR System (for general qPCR applications), CFX Opus 384 Real-Time PCR System (for high throughput labs), and CFX Opus Deepwell Real-Time PCR System (for larger volumes up to 125 µl), Bio-Rad qPCR instruments are distinguished by the use of a unique optical shuttle system. The optics shuttle scans above the sample plate, individually illuminating and detecting fluorescence from each well while avoiding cross talk. In taking these measurements, the optics shuttle is centered above each well with every scan. Consequently, there are no variations in light paths. All wells experience identical light paths and intensities, effectively eliminating the need to normalize to a passive reference and requiring only baseline subtractions of individual well fluorescence data. This further minimizes the amount of data processing that will have to be performed by the qPCR software.

ADVANCING TRANSLATIONAL RESEARCH

CFX Opus Systems are powerful and flexible instruments with demonstrated potential for advances in translational research and testing of biopharmaceutical products. The CFX Opus Systems' reduced-mass sample block heats and cools faster than standard blocks resulting in improved thermal uniformity and minimization of edge effects. This is crucial in protein thermal shift assays that measure changes in the thermal denaturation temperature and provide information on protein stability under varying environmental conditions. Efficient and specific target amplification hinges on the determination of optimal temperatures for primer annealing. By allowing users to program temperature gradients with a difference of up to 24°C across the reaction block, with each gradient zone experiencing optimal temperature uniformity and reproducibility, CFX Opus Systems provide efficient assay optimization in a single experiment. This helps minimize wasting samples and reagents while saving valuable research time.

The development of relevant model systems and the detection and testing of potential therapeutics

and biomarkers are highly crucial for progressing potential therapeutic methods from preclinical to clinical trials. In this effort, the objective is to make the transition from preclinical to clinical trials faster, more efficient, and effective. The innovative shuttle optical design of the Bio-Rad CFX Opus qPCR instruments succeeds in this objective and offers various benefits starting with the availability of a greater number of channels for multiplexing without the requirement of a reference dye. The CFX Opus 96 Real-Time PCR System facilitates true five-target multiplexing as it can distinguish up to five targets in a single reaction well. This ability to multiplex is beneficial for the detailed analysis of stem cells, differentiation pathways, and gene expression (without ROX) studies. Biological reactions and signal networks can thus be monitored in real time. These advantages also come in handy for biomarker analysis that can be used to evaluate pluripotency, lineages, and differentiation. CFX Opus 96 System also includes one channel with an LED-filter photodiode allowing for Fluorescence Resonance Energy Transfer (FRET) experiments, further expanding experimental options.

The integration of CFX Maestro Software with PrimePCR™ Assays aids researchers in quickly learning how to set up a plate, analyze samples, acquire data, interpret, and visualize the experimental results. The software further allows for combining data from multiple plates into a multi-plate gene study analysis for rapid screening of large numbers of samples. Visualization tools such as hierarchical clustering and color-matched expression levels further simplify the reliability of data interpretation for the identification of individual targets or clusters for detailed investigation. These advantages also translate toward greater performance and efficiency in other applications including viral load titer studies, mutation detection, residual DNA testing, biodistribution/persistence in biologic therapeutics, and copy number variation—to name a few. Accompanied by an easy-to-use user interface, built-in adaptability, increased sensitivity and dynamic range for flexible formats, and optimized integration with automation for high-throughput labs, the Bio-Rad qPCR systems enable innovation at the highest level in translational research.

SETTING A NEW STANDARD

qPCR assays are a widely used method for rapid and sensitive nucleic acid determination and quantitation in biological samples with diverse applications in multiple fields of research. Bio-Rad CFX Opus Systems use a novel optical shuttle system facilitating greater accuracy, reliability, and consistency in qPCR analyses. Supported by an extensive statistical analysis tool in the CFX Maestro Software, Bio-Rad's optical shuttle-based qPCR instruments present a comprehensive and innovative solution for greater advances in translational research and testing of biopharmaceutical products.

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