DATA IN HARMONY: MODERN APPROACHES TO QPCR

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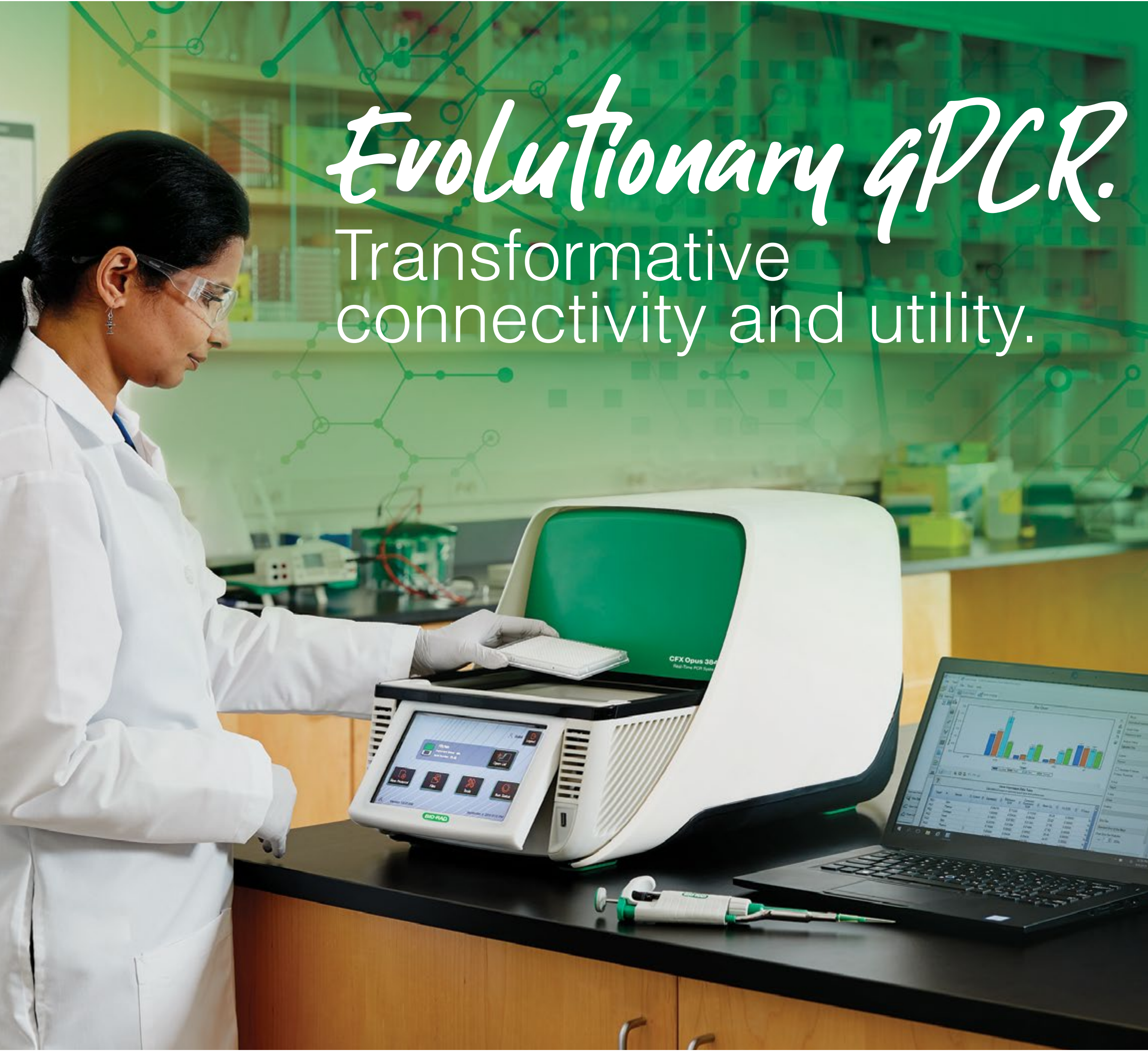
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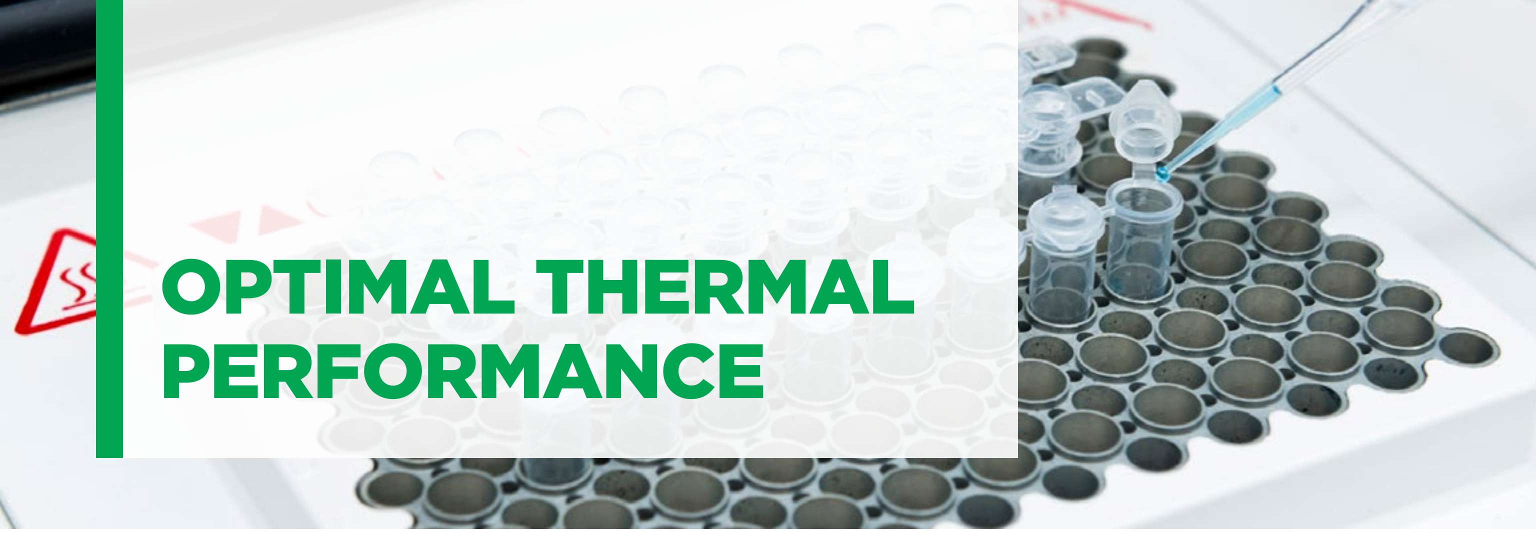

OPTIMAL THERMAL PERFORMANCE

Scientists use quantitative polymerase chain reaction (qPCR) for a variety of sensitive applications. From multiplexed gene expression profiling to infectious disease diagnostics, researchers require reliable qPCR instruments.

Some thermal cycler blocks may produce suboptimal results, as issues with thermal uniformity affect the rate and efficiency of a reaction. Because small amounts of genetic material are exponentially amplified, small perturbations can lead to large problems. Instruments that precisely control reaction conditions are essential for highly sensitive assays where a specific annealing temperature is required. Reactions may fail or the accuracy of the quantified data may be brought into question if the temperature deviates from the ideal.

Around the Block

An important consideration for qPCR machine performance is the time it takes to heat or cool the block with each temperature change. How quickly an instrument changes temperature is an important determinant of the duration of an experiment. Instruments must balance the time it takes to change temperatures with uniformity. The temperature in each well should stabilize quickly across the block so that each sample experiences the same reaction conditions. This task is more complicated than it seems, as speed often negatively impacts thermal uniformity.[1] Blocks with reduced mass heat and cool more quickly, shortening the cycle time. However, blocks with higher mass maintain temperature uniformity better than those with minimal mass. Heat transfer from the block to the sample should also be rapid for high efficiency generation of target DNA.

Peltier elements control heating and cooling throughout the reaction cycles in many instruments. These devices tend to be cooler around their edges than in the middle, which may create temperature variation throughout the block, particularly at the edges and surface of the block.[2] Insufficient heating around the block edges (the edge effect) and hot and cold spots throughout create experimental variability.

Impact on Data

The edge effect can lead to insufficient template DNA melting, especially when the template has a high GC content. This decreases nucleic acid amplification, leading researchers to underestimate the amount of target in their samples. Additionally, undershooting the temperature may cause amplification of unwanted DNA.[3] In this situation, primers may bind their target insufficiently or they can anneal to sequences with weak sequence homologies. Hot spots in the reaction block may inactivate the polymerase, which generates false negative results.

Temperature variability also affects the melt curve. Identical qPCR amplicons in separate wells may appear to melt at different temperatures. Researchers then incorrectly interpret these results to mean that their primers are amplifying more than one product. They waste precious time and resources optimizing primer sequences and annealing temperatures to correct a problem that is not real.

Virus diagnostics

Increasingly, scientists perform qPCR for molecular diagnostics. In particular, widespread use of qPCR to diagnose SARS-CoV-2 highlights the practicality of the technique for infectious disease testing. qPCR delivers test results quickly compared to previously-used methods. Clinicians apply the technique to easily determine if someone is infected with a specific disease, while researchers can use qPCR to quantify viral loads and monitor therapy responses.

Issues of speed and uniformity are especially important for clinical sample testing because quick and reliable results allow physicians to diagnose, treat, and stop the spread of communicable diseases. Viral diagnostic experiments are especially sensitive to changes in temperature. Different virus strains can be very similar, so incorrect primer binding due to temperature irregularities causes inaccurate diagnoses. Scientists in this field should seek out high-quality machines that monitor and tightly control temperature across the block, ensuring reproducible and accurate results.

References

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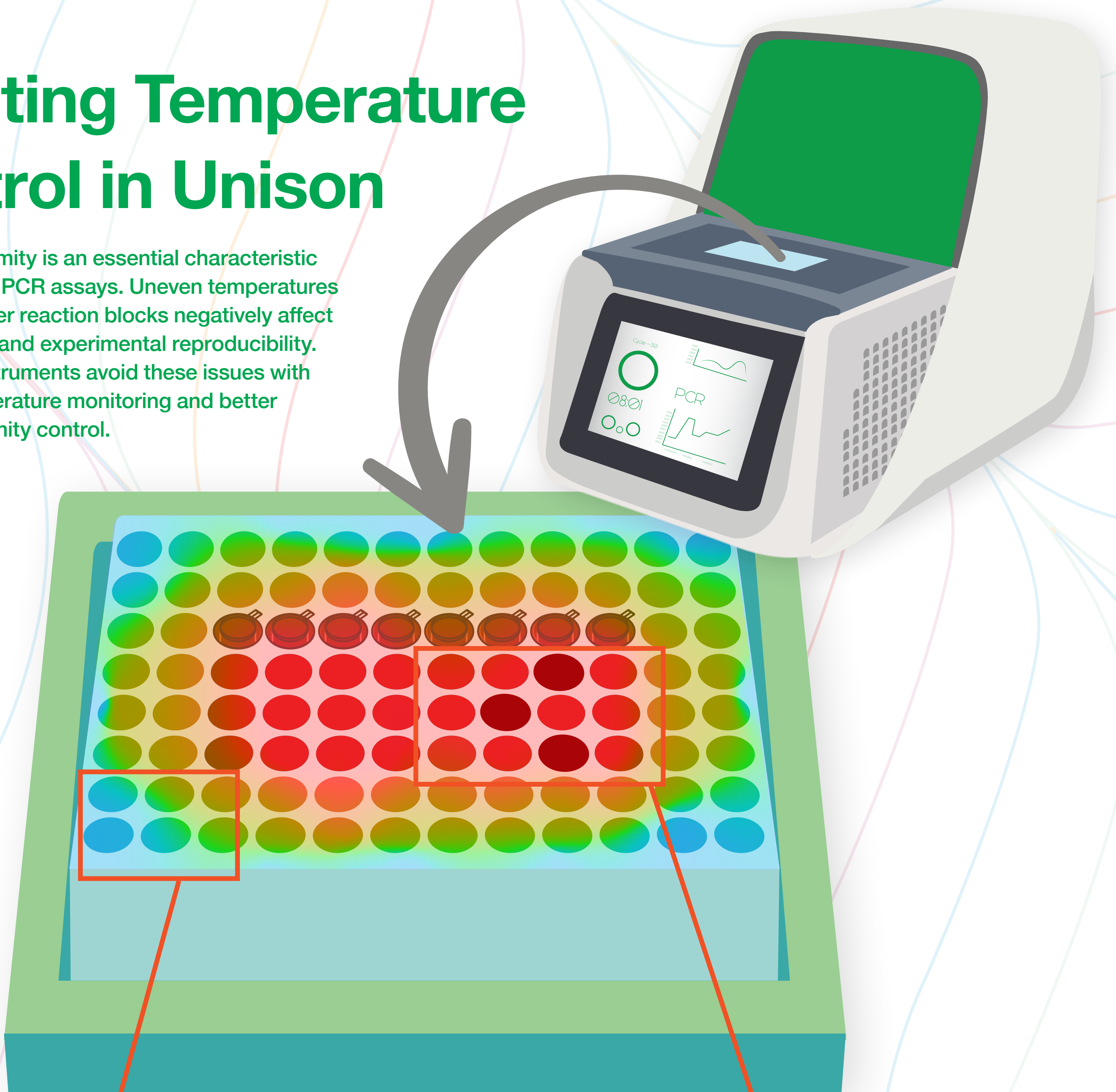
Creating Temperature Control in Unison

Thermal uniformity is an essential characteristic of successful qPCR assays. Uneven temperatures in thermal cycler reaction blocks negatively affect data accuracy and experimental reproducibility. New qPCR instruments avoid these issues with effective temperature monitoring and better thermal uniformity control.



Speed Vs. Uniformity

qPCR machines with reaction blocks of minimal mass quickly change temperature throughout a run, but these machines may lack thermal uniformity because of the thin metal. High mass blocks maintain consistent temperatures at the expense of speed. Instruments that balance speed with uniformity produce the most accurate results.

Out in the Cold

Cold spots and edge effects undershoot reaction temperatures, leading to decreased primer annealing and under-estimated target quantities. Cold temperatures also cause off-target primer binding to sequences with lower homology.

Too Hot to Handle

Hot spots denature and inactivate the polymerase, causing false negative results.

ORCHESTRATING DATA CONNECTIVITY

In modern society, countless individuals are connected electronically; cell phones are mini computers and artificial technology controls basic functions in many homes. But when it comes to laboratory technology, many scientists find themselves sent back in time. Out-of-date computers hooked up to modern lab equipment inefficiently store bits and pieces of data that await analysis. Researchers should leave old technology behind and ready themselves for the future by storing and analyzing data on a cloud-based platform.

Convenience in the Lab

Instruments that are connected to the cloud grant researchers freedom from the bench. Some machines even link to the cloud directly, without the need for a dedicated computer that is connected to the internet. By accessing the cloud with any web browser, researchers plan and manage experiments anywhere and at any time. They check the status of an experiment as it runs from any computer, and results often upload automatically when an experiment is complete. Scientists can also analyze their data from anywhere without transferring files via unsecure USB drives.

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Data Management

Cloud-based computing and data storage help researchers move away from the confusion caused by handwritten lab notebooks and poorly labeled spreadsheets and graphs. Many cloud storage platforms for scientific data provide tools for data analysis. Researchers are beginning to transition their data storage, organization, analysis, and figure development to these web-based platforms.

Another major benefit of cloud data storage is that it simplifies collaboration. As research teams spread across the globe, scientists easily share their uploaded data with lab members and external collaborators. Keeping data in the cloud also enhances reproducibility because researchers can share virtual lab notebooks with collaborators and directly connect instruments to the cloud to automatically record experimental conditions.

Cloud services safeguard data from accidental loss or from unauthorized users making changes by providing secure and redundant data centers. Within a lab's cloud platform, scientists can generate multiple user profiles with individual usernames and passwords to further organize data and keep results secure.

Solution for Big and Small Data

Modern molecular instruments produce mountains of data thanks to multiplex capabilities and single cell and whole genome analyses. Also, as the cost of sequencing continues to decrease, the amount of data generated will only increase. Researchers need cloud-based strategies to keep track of this information. Whether the dataset is small, such as that from a qPCR experiment analyzing the expression of a handful of genes, or large like those from single cell sequencing projects, researchers benefit from working with their data in the cloud.

In particular, cloud-based software simplifies biomedical research. Researchers in labs across the world can remotely view large next-generation sequencing datasets that are uploaded to the cloud and analyze them for single nucleotide polymorphisms (SNPs), mutations, and new gene variants. Scientists can also integrate results from other technologies, such as mass spectrometry and imaging, to better understand biological mechanisms for diagnostics research.

MARCHING TO THE BEAT OF A DIFFERENT DRUM: ALTERNATIVE QPCR APPLICATIONS

QPCR is no longer employed only by molecular biologists in research laboratories. Scientists in diverse fields use the technique for a variety of applications. Therefore, qPCR reagents and instruments that take up minimal space and are easy to use are desirable in these industries.

Managing Microbes

Microorganisms are everywhere, yet they are invisible to the naked eye. Scientists in many fields track microbes to either eliminate them or support their growth. Traditionally, scientists analyzed microbe populations by counting colonies on a plate or growing cells in liquid culture. This approach lacks species specificity, only estimates the number of cells present, and takes days to obtain results, whereas qPCR methods detect different species and strains in a single multiplexed experiment, accurately quantify the amount of cells in a sample, and take mere hours to complete.

qPCR is now the method of choice for microbial monitoring in the food safety and processing fields. Scientists use species- and strain-specific primers to quantify and identify food-borne pathogens, including small populations that would be hidden by more dominant ones in tube or plate cultures. Additionally, scientists perform quantitative reverse transcription PCR (RT-qPCR) to track microbial gene expression. In one example, researchers working to improve wine production measured gene expression by qPCR of acid stress response genes in lactic acid bacteria.[1] The scientists employed the data to enhance the viability of these desirable microbes in wine.

Scientists also use qPCR to target microbial genes in environmental samples with kits that easily extract DNA or RNA in the field. Researchers working to reduce water scarcity analyzed the safety of roof-harvested rainwater with qPCR to identify pathogens in their samples.[2] Microbial contamination is also a concern in oil fields. Water injections that stimulate oil extraction can also introduce hydrogen sulfide-producing microorganisms that generate toxic gas, lower oil quality, and corrode pipelines. Scientists monitor oil field conditions by processing samples in the field and running experiments with small-scale qPCR instruments.[3]

> "qPCR is no longer employed only by molecular biologists in research laboratories. Because of their various backgrounds, scientists prioritize qPCR technology that is simple to operate."

Supporting Agriculture

Agriculture researchers face the enormous challenge of feeding the growing world population. These scientists improve the nutrition and output of crops and livestock through genetic means. They use qPCR to analyze animal and plant gene expression. Additionally, qPCR simplifies genotyping by showing the absence or presence of transgenes and distinguishing between heterozygotes and homozygotes based on gene dosage. Easy genotyping is also important to track adventitious presence—when trace amounts of an unwanted agricultural biotech product ends up in a field population or food supply.

qPCR for All

Because of their various backgrounds, scientists prioritize qPCR technology that is simple to operate. Drag and drop tools, intuitive interfaces, kits that extract nucleic acids in the field, standalone instruments that use the cloud for data storage, analysis, and sharing all promote qPCR use across disciplines.

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