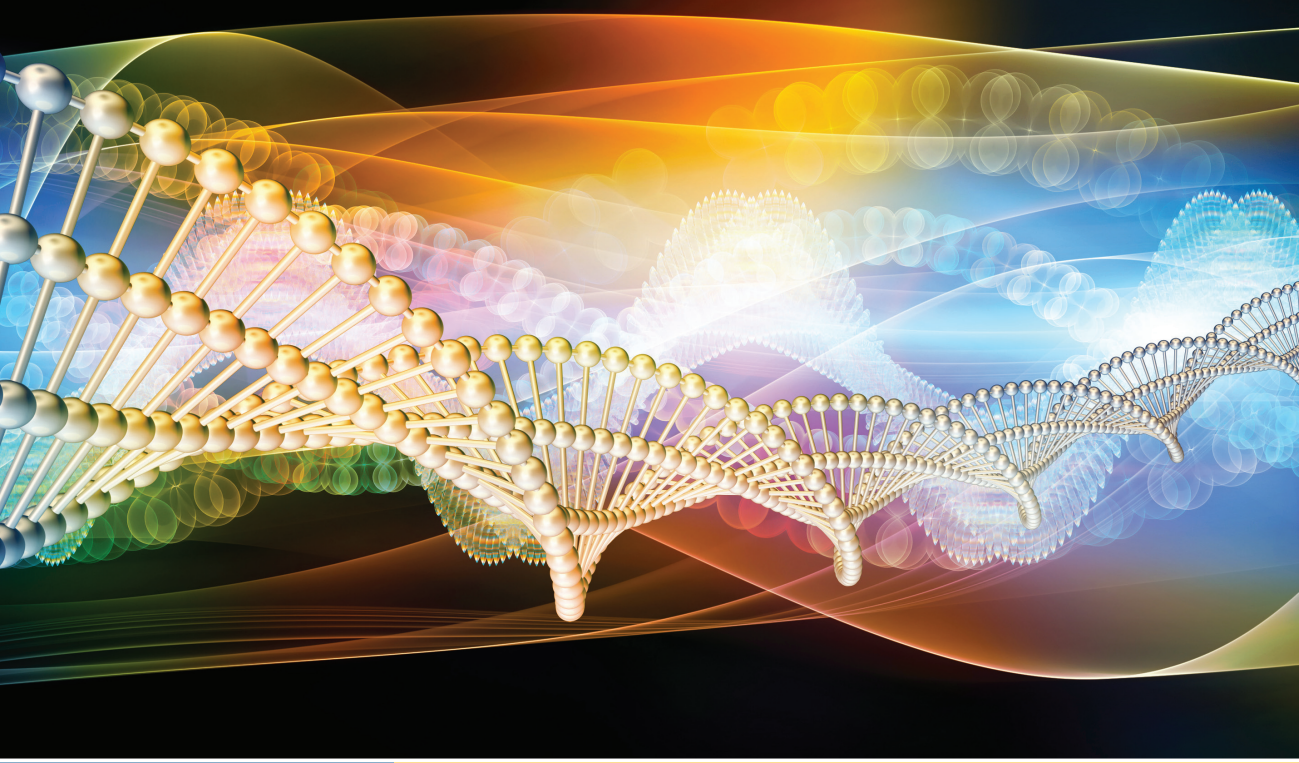
Third Edition

PCR Technology

Current Innovations



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- 40 cycle qPCR in under 10 minutes.
  - With ultra-low thermal mass & high conductivity **xxplates**.
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  - Highly accurate IR Sensors measure temperature.
  - Patented Algorithm adjusts temperature 100 times per second.
- Exceptional temperature uniformity.
  - Better than 0.3^{o} C during cycling.
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  - Programs the run the way you think.
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10 Ultra-High-Speed PCR Instrument Development

Nick Burroughs and Emmanouil Karteris

CONTENTS

10.1 INTRODUCTION: PCR TEST SPEEDS

Polymerase chain reaction (PCR) testing has come a very long way since its inception by Mullis et al.[1–3] way back in 1983. PCR is now regarded, by most bioscientists, as the "gold standard" method for sample analysis based on genetic markers. The ability to replicate a few strands, or even a single strand, of specific genetic material many times, thus enabling the identification of the marker, has become the core technique for a generation of bioscientists.[4]

Initially, PCR tests required postamplification analysis by methods such as gel electrophoresis with ethidium bromide detection. Subsequently, "real-time" PCR (or qPCR) was developed, which combines the PCR process with a fluorescent probe or a DNA-binding dye detection in the same reaction vessel. This dramatically enhanced the original PCR process giving faster results with more accurate quantification and reduced risks of contamination. As the process has evolved, the applications for it have been increasing exponentially with PCR now forming the basis of thousands of tests, with applications ranging from infectious diseases to paternity identification, and from forensic analysis to food processing. Figure 10.1 shows the numbers of published manuscripts over the last few years giving an indicative measure of the research effort in the PCR field.

However, even at our time of writing, a typical 40-cycle PCR can take around 2 h to complete and that time has struggled to keep pace with other advances in the area. So, some of the potential benefits of this extraordinary process remain limited by the speed of the test. The speed of a PCR test is not just important for its own sake but also because there are applications for which the time to deliver a test result could mean the difference between profit and loss, or even life and death. In a research laboratory where the researcher will have other work to do while they wait for their PCR

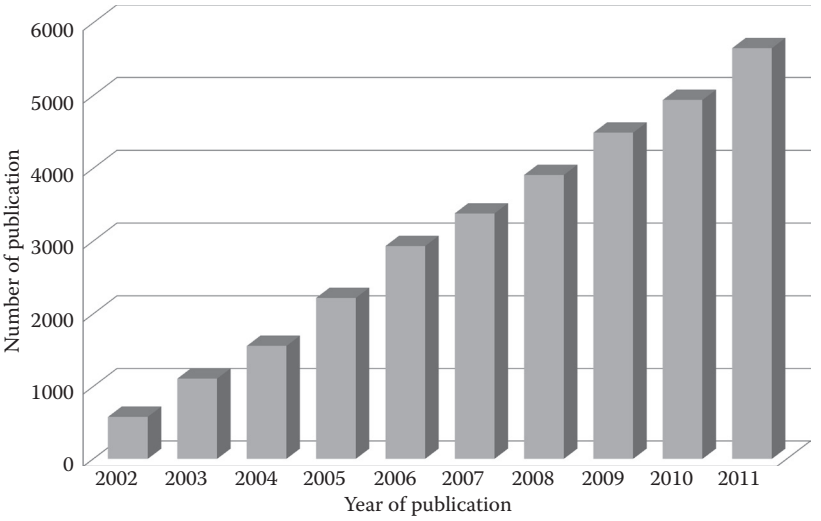

FIGURE 10.1 Manuscripts published in PubMed using real-time PCR technology over the last 10 years.

test to complete, 2 h could be regarded as an inconvenience that slows their progress; however, in a hospital, the difference between that and, for example, 10 min can save lives.[5]

A simple daily example of the importance of speed in diagnostic applications is the issue of MRSA (methicillin-resistant *Staphylococcus aureus*) identification. Patients entering hospital for a planned procedure are routinely tested for MRSA a week prior to their admission; blood samples are sent to a central clinic and typically the results take a few days to process; the actual time required for sample preparation and test is about 5 h. However, for emergency admissions, this process is too long; so, the patient is admitted with the risk of carrying the infection and therefore with the potential to infect others. Having a test that could be administered either at admission to the hospital, or even in an emergency vehicle prior to arrival, and would only take a few minutes would provide a real benefit. It is similar within an industrial setting; if a PCR test is being used to monitor a food process line for contamination, a 10-minute test could be used as an in-line process, whereas the current tests are typically applied retrospectively to daily batches meaning that the potential for costly wastage is much greater.

It is not just applications for which time is critical, that benefit from greater speed. It is far more efficient if a patient can be tested and treated on a single visit to their doctor rather than having to make a journey to the clinic to provide a sample for the test, and then return a week later to receive the results and potential treatment. A single visit would result in the time required away from work being halved, less miles being traveled, more efficient use of health care resources, faster treatment, and reduced patient stress. To make a single visit viable requires these clinic-based tests to provide their results within 30 min. Such locally administered, "while you wait" tests, referred to as "point of care" (POC) tests, can include not only tests for specific diseases but also for the effectiveness of a particular treatment on the patient. Referred to as "companion diagnostic tests" (CDx), these tests can help identify the most appropriate treatment and dosage.

10.2 WHAT LIMITS CURRENT PCR TEST SPEEDS?

The PCR process requires that the test samples are cycled through a temperature profile; a typical profile will see samples cycled between 95°C, 55°C, and 72°C, multiple times. The time taken to change the temperature of the samples between these levels is a key determinant of the speed of the process and thus of the length of a test. Tests often require a number of samples to be cycled simultaneously; for example, for comparison to each other or as process controls, each sample needs to experience the same conditions if these comparative results are to be valid.

The design of many PCR instruments relies on conductive blocks to connect the heating or cooling source(s) to the test samples; heat naturally flows within the blocks to remove any temperature gradients and so, it should, over time, deliver the same conditions across all the test samples. However, this will always be less than perfect due to the variability of cooling across such a block; uniformity of the block temperature is vulnerable to greater heat losses on the edges and surfaces that tend to distort the thermal distribution.[9] The conductivity of these blocks also affects the rate of heat flow and thus affects the thermal uniformity of the samples. That conductivity is directly related to the raw material and its thickness; thicker blocks have better conductivity; however, they also have higher thermal mass. The larger the thermal mass of the block, the greater the amount of heat that needs to be transferred and the longer this will take.

To heat and cool the system, heat needs to be driven in and out of the block. The faster the heat is driven in or out of the system, the less time the conductive block has to even out the temperature distribution and maintain the thermal uniformity. Ultimately, such a system can only maintain its thermal uniformity if the rate of change of the temperature is slower than the time taken by the conductive block to even out the temperature. So, in these type of systems, the need for uniformity of temperature is in direct conflict with the desire for speed; they can deliver one feature or the other but not both. The faster these thermal block systems are driven, the greater the variation of temperature across the test samples. Even the most highly conductive silver blocks are limited to rates of change of temperature of <3°C/s if they are to maintain acceptable conditions across all the test samples.

Systems of this type usually use one or more Peltier cells to provide both the heating and the cooling to the heat-transfer block. Peltier cells can be used to both heat and cool units depending on the direction of current flow. While the control of the heat output of a Peltier cell can be regulated quite precisely, the thermodynamic design of the rest of the system limits its performance. Control of these systems is problematic since a temperature gradient is required to force the temperature flow from the heating source to the test sample; heat flows from high temperatures to lower temperatures; the greater the difference, or gradient, the faster the flow. To achieve quick cycle times, big temperature gradients are applied to the block, which can lead to samples overshooting or undershooting their target temperatures.

These physical limitations of using a "passive heating" method (heat-transfer block) for the PCR process were recognized by BJS Technologies Ltd. As a result, they developed an innovative concept for measuring the temperature of the sample and then providing additional heat where it was needed. It was this technology, called xxpress™, which was used to create the xxpress real-time thermal cycler.

xxpress is an "active heating" methodology that precisely delivers heat to control the temperature of the test plate. It does this by accurately measuring the temperature of the sample and then precisely controlling the amount and location of the additional heating. Low thermal mass is an advantage in a system such as this as it reduces the reaction time from the input of energy to deliver a change in temperature, thus enabling better control. The combination of active heating and low thermal mass works together to enable much faster rates of temperature change while still maintaining uniform temperatures across the sample area. With heating rates of >10°C/s, the system can achieve a thermal uniformity of ±0.3°C. To achieve this performance, the software control algorithm is measuring the temperature of the samples 100 times/s and is calculating where and how much heat to apply.

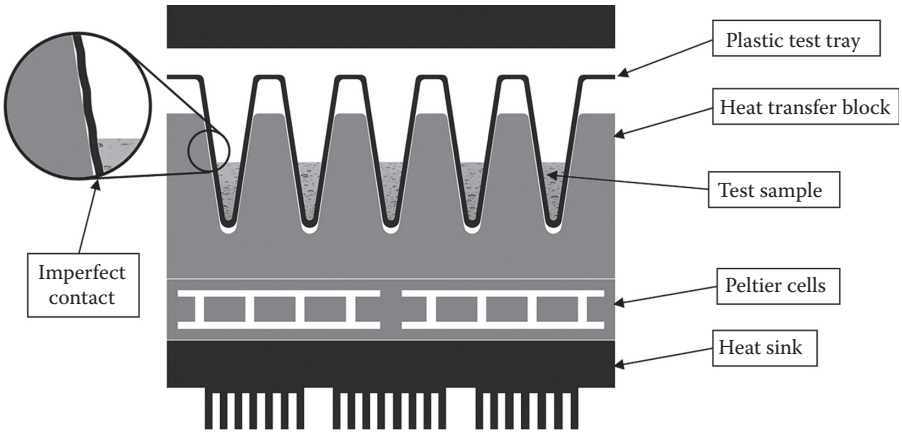

FIGURE 10.2 The layout of a traditional PCR thermal cycler with heat-transfer block showing the major components.

Another key limiting factor to the fast and accurate control of the thermal profiles of the test samples is how those test samples are thermally connected to the heating source. In traditional thermal cycler designs, a thin plastic sample tray, or microtiter plate, sits over the heat-transfer block (see Figure 10.2). This is a disposable test plate and is replaced for every experiment. It makes physical contact with the heat-transfer block and heat flows from the block through the plastic material to heat the sample. The contact from the block to the plastic is not perfect; plastic has a different expansion coefficient to metal and plastic is an insulator so that the heat flow to the samples is restricted and is uneven.[6–8] This leads to a time lag for the sample to achieve the same temperature as the heat-transfer block and that time lag varies from cell to cell depending on the amount of contact between the plastic and the block well. Time lags for conventional PCR block systems can typically be 10 s and while suppliers adopt different strategies to minimize the effects, this factor adds time into each thermal step because the system needs to ensure that each test sample has reached the desired target temperature before the required incubation time can be started. If the test sample could be brought into more intimate contact with the heating source, then the time lag would be shortened, the uncertainty would be reduced, and cycles could be faster.

10.3 HEATING AND COOLING VERY QUICKLY BUT UNDER CONTROL

In the previous sections, the need for faster thermal cycling was identified. In the following sections, the technical challenges that have to be addressed when designing such a system will be described.

The issues around thermal mass and temperature control can be examined by consideration of the physics of heat management within a heating control system; this is essentially what a PCR thermal cycler is. Heat is provided from a source, it is used to raise the temperature of a group of samples to a defined point, and hold them there. There will be heat lost continually from the system by cooling because the temperature of the system is above ambient. There are two states to consider for the system; steady state where the goal is to maintain the temperature of the system and ramping where the temperature is being actively increased or decreased.

At the incubation temperatures, a steady thermal state is required for all samples. In a steady state, the aim is to keep the samples at a chosen temperature and so the points *A* and *B* shown in Figure 10.3 should ideally be the same temperature. Assuming that the heat-transfer properties from the block into the sample wells at points *X* and *Y* are the same, then to maintain a steady state, the temperatures at *X* and *Y* should be the same. But since *X* is located at the edge of the block, the cooling effect is greater than *Y*.[9] This results in a temperature difference between *X* and *Y*. One way to minimize that difference

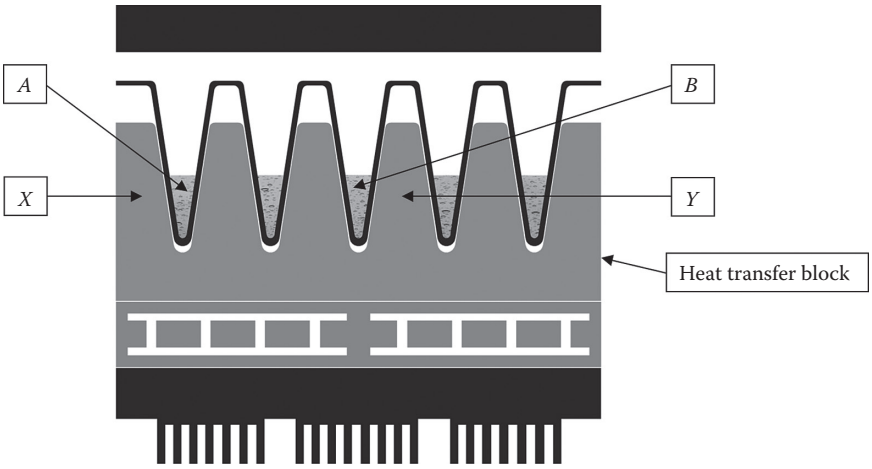

FIGURE 10.3 Schematic figure showing a section through a traditional thermal cycler.

is to ensure that the thermal resistance between *X* and *Y* is very low so that the heat flows easily between these points and the temperature is equalized. The heat-transfer block in a PCR thermal cycler is designed to provide low-resistance path; the precise design will depend on the required accuracy of the instrument that will determine the maximum value for the temperature variance across the plate.

In these systems, the greater the thermal resistance of the construct material, the thicker the heat-transfer block needs to be to achieve the thermal uniformity. The thicker the block, the greater the thermal mass, requiring more heat to be moved in or out for the required temperature change. This thermal inertia also slows down the response of the system, making it more difficult to control and leading to greater under- and overshooting of the temperature. So, the thermodynamics of such a system is in conflict; a higher thermal mass is required to ensure that the temperature across the samples is uniform and a lower thermal mass is required to provide accurate control of the plate temperature. To reduce the temperature variation across the plate, the ramp rates need to be low, but there is a desire to ramp the temperature faster to deliver the test results more quickly. Any system that uses a heat-transfer block or heating plate in this way will rely on compromise. A number of systems have been developed that incorporate ways to enhance the heat distribution; the ECO qPCR system from Illumina uses a stirred fluid inside the thermal block and to ensure sample uniformity, they also limit the size of the block. However, these modifications have only made small differences and they do not change the fundamental thermodynamic challenges.[10,11]

Other models, such as the Rotorgene from Qiagen, do not use heat-transfer blocks at all; they rely on hot air to heat the samples. Spinning the samples through this heated air gives good thermal uniformity; however, while faster than heat-transfer block systems, these instruments thermal ramp rates are still limited. The walls of the sample containers are made of insulating material and limit the rate of heat transfer.

An alternative approach was adopted by BJS, who aimed to remove these limitations by using an "active" control process. The concept of an "active" heating system is to divide the area to be thermally controlled into smaller areas or zones. Each of these zones has its own temperature measurement and its own heating system. For each zone, the control system determines how much heating is required to achieve a specific temperature change. The heat is then provided and the temperature is re-measured. The control system uses the ratio between the resulting temperature change and the initial delivery of energy to determine the requirements for the next heating cycle. While this is a simple concept to understand, the complexity of converting this concept into precise instrument performance took the company over 10 years of development. It was also deemed desirable to measure the actual temperature of the sample, or at the very least, something that was intimately linked

to it, because traditional systems rely on measurements of the temperature of the thermal transfer medium, the block, or heated air, and these can be quite different from the sample temperature. The following sections contain descriptions of the issues that were encountered during the development of this system and the solutions that were developed.

10.4 TEMPERATURE MEASUREMENT

To deliver an active temperature control process as described earlier, the initial temperature of the plate needs to be established. The measurement of any temperature is a challenge since it is difficult to measure reliably and accurately without affecting the temperature of the material being measured. The xxpress thermal cycler system was developed with the aim of achieving a uniformity between test samples of better than 0.3°C. To control and deliver this, the system must be able to measure test sample temperatures to an accuracy of better than 0.1°C and to do this very quickly to allow precise control. This is a high level of accuracy for such a temperature measurement; contact and noncontact measuring systems were considered.

Contact measurement systems were quickly discounted; placing sensors directly onto a test consumable would add too much to the price of each test and connecting the individual sensors would also be challenging. (At the desired level of accuracy, the difference in the resistance of a connection between operations could be enough to invalidate the results.) A number of methods of placing thermal sensors in contact with the test plates were investigated; however, each method proved to suffer from variations at the contact interface. In addition, there is a potential for the contact to materially change the test plate temperature.

Noncontact sensors that measure the infra-red radiation emitted from a sample seemed to be the most realistic option. Using this approach would not affect the consumable plate or tube, there would be no connections to make or break for each sample, and the same sensors would be used to measure every sample so that this should improve consistency. However, a method of measuring to a higher accuracy than is typical for these sensors had to designed. Working with the U.K.'s National Physical Laboratory (NPL), a specialized calibration method was developed that determines a range of coefficients that can be applied to each of the temperature measurements to achieve the required repeatability and accuracy. Critical to the selection of these sensors were their response times. The aim was for each sensor to make 100 measurements every second as the faster this feedback loop could work, the better would be the control of the plate temperature.

The area of the test plate was subdivided into sections that were measured by a single sensor and could be controlled by the current paths that were available. A number of sizes of array and of thermal sensor were then tested to ensure that the system had sufficient control and that any variation in the temperature over the area covered by the array was much smaller that the accuracy required in the measurement.

From the number of options tested, a design consisting of nine sensors on a 3×3 grid with nine defined heating zones on the test plate proved to give the best uniformity of temperature across the chosen test plate size. This decision also influenced the design of the heating system that is described in the following section.

One of the benefits of this type of temperature measurement system is that in addition to providing the information required by the system to control the temperature of the samples, it also provides a record of the actual profile of temperatures of the samples proving that they have been controlled in the way that was expected. This data provides, a verification log of the actual thermal profile experienced by each of the test samples and is attached to the experiment's data file.

10.5 ACTIVE HEATING AND COOLING

Active heating control requires the ability to direct heating to specific areas or zones of the plate in preference to others and to precisely control the amount of heat delivered. After reviewing

different heating approaches, the only practical solution that could give the precision of control and the speed of ramping required for the new system was electrical resistance heating. Electrical resistance heating is the conversion of electrical energy into heat energy when an electrical current flows through a conductor such as a metal. It has two key properties that make it ideal for this technique. First, heating occurs at the atomic level within the material and is directly proportional to the current flowing through the material. This way, it effectively heats from within and the heating is in precise and direct correlation with the electrical current that was passed through it. The second property is that, in a metallic conductor, the shortest path through the conductor has the least resistance, and hence, the greatest current flow. In a situation where heat is proportional to current flow, where the current flow is greater, so is the heating, thus, mapping the current flow through the conductor can be used to control the heating. The technical challenge was then to balance the number of current paths and the resistance of the heating element to deliver the control and accuracy that a PCR system requires.

After a significant investigation requiring thermal modeling and the testing of enumerable samples, a range of relationships were defined that were then used to create a single heating plate (see Figure 10.4) and to map a range of current paths through that plate. One of the key factors in the design was the relationship between the electrical conductivity and thermal conductivity of the plate. The better the thermal conductivity of the plate, the faster the heat can flow to correct any minor variations in the plate temperature and so, fewer heating zones will be required. In a metal, the better the thermal conductivity, the lower the electrical resistance and so, the greater the current that must flow through the plate to provide the required heating. Thus, the choice of material for the consumable plate affects the design of the power supply, the rest of the heating circuit, and the connections within it.

The optimum balance of all the factors described above led to the creation of a simple, thin aluminum plate with six electrical contact fingers, as shown in Figure 10.4. It has a very low thermal mass when compared to the traditional PCR systems and so, it can be heated and cooled much more efficiently, with ramp rates of >10°C/s being possible. Mapping the current through different combinations of these six contact fingers provides a sufficient range of heating paths to enable both the accurate control of the heating of the plate and also the ability to maintain a thermal uniformity of better than 0.2°C. Finally, the resistance of the plate, while quite low was still high enough that an electrical circuit could be practically constructed around it to drive it.

Heating is only one part of the problem when designing a thermal control system for a PCR system; the system also needs to be able to cool to complete a thermal cycle. This was achieved in xxpress by directing jets of cool air onto the base of the thin aluminum plate. These jets are driven by individual, continuously variable pumps so that control of the cooling can also be adjusted to match the cooling required in each area of the test plate. This cooling design can produce rates of cooling of greater than 15°C/s. However, the response time of this type of cooling system is slower than the resistive heating system due to the inertia of the air movement and so it cannot provide the same precision as the heating system. To achieve the same thermal control and uniformity for cooling as for heating, the resistive heating system is used to provide fine control during the cooling process. It provides small amounts of heat into the cooler parts of the test plate to slow their cooling to match the desired rate.

10.6 THERMAL CONTROL ALGORITHM

In the earlier discussions, the ability to provide “active control” of the thermal system is assumed once the physical parameters have been optimized; however, this proved to be one of the key challenges of the development. The system has multiple degrees of freedom and required considerable analysis to develop a control theory and subsequent algorithm.

There are a number of current paths that can be chosen to heat the plate; each path will heat a number of zones, not just a single zone. So, the control algorithm is required to make choices on

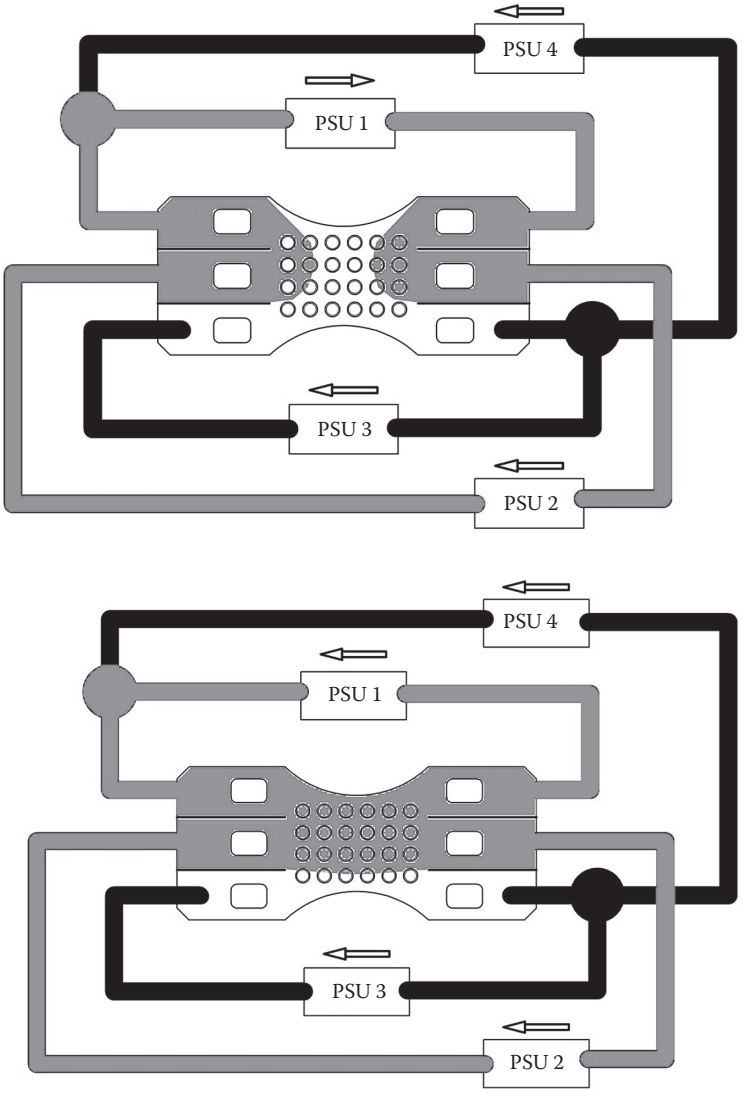

FIGURE 10.4 Two examples of an xxpress™ sample plate showing different current flow patterns.

how best to raise the temperature of a particular zone without detrimentally affecting the temperatures of other zones. Within the same time slice, all the zones will also be changing temperature as they will be above the ambient temperature and subject to cooling; that cooling rate will vary across the plate; the problem is a complex one.

The solution that was developed combined a set of rules for decision making and some high-level mathematical control theory. The decision-making rules guided the algorithm as to the best strategy to use to affect the temperature of the zone based on the desired change. Then, the control theory would calculate the correct electrical energy to deploy to deliver the required change. Finally, the sampling speed of the system was balanced with the thermal inertia of the consumable to give the accuracy required. In the format of the xxpress thermal cycler, that sample speed sees the control loop measuring, calculating a reaction, and delivering the next heating action 100 times every second.

10.7 SAMPLE CONTACT WITH THE HEATING SOURCE

Until this point, the discussion has been focused on controlling the temperature of a test plate or a thermal block but the real requirement of a PCR system is to control the temperature of a small test sample, typically 0.5–40 μL of reaction mix. In traditional PCR instruments, these test samples are contained within multiwell "micro-titer" trays (disposables). These are molded from polypropylene and have a typical wall thickness of 0.2 mm. The individual test wells fit snugly into a mating form on the heat-transfer plate so that heat can travel from the thermal block through the polypropylene to the test sample. However, this process is far from efficient because the walls of the microtiter tray touch the heat-transfer plate intermittently and the thickness of polypropylene used has significant insulating properties. This means that the temperature of the samples generally lag the temperature of the transfer block, often by over 10 s and the degree of temperature difference can vary from sample to sample.

In an effort to minimize this effect, BJS identified a method for coating the aluminum consumable directly with polypropylene so that the plastic would always be in excellent contact with the heat surface. The coating of polypropylene is only 0.01 mm thick that is 20 times thinner than a microtiter plate, allowing more efficient conduction of heat. In addition, they designed the test wells to be flat bottomed to maximize the contact area with the aluminum plate. These critical design steps enable the test samples to follow the temperature of the test plate very closely, resulting in typically less than 0.5 s time lag, and this lag is consistent from well to well. The time lags for all the wells on the tray are over 20 times better than traditional designs. This improvement not only speeds up the reaction time as the system does not need to wait for the sample temperatures to stabilize, but it also provides a much more precise thermal profile for the test samples. As has been shown, PCR experiments give more dependable results when temperatures are brought more quickly to the target temperature.[12]

10.8 UNDERSHOOT AND OVERSHOOT

As previously discussed, the thermal response of a typical PCR system limits its speed and controllability. Thus, as the target temperature of the thermal cycler is approached, the control system has difficulty in delivering the desired temperature both quickly and precisely. As a result, such systems either overshoot or undershoot the desired temperature. Overshooting leads to the samples experiencing temperatures in excess of those planned, often significantly higher, which can damage the sample. Undershooting results in the samples arriving at the desired temperature more slowly than required; the samples do not reach the desired temperature for the requisite time, which may lead to an incomplete reaction step. Both of these issues can be avoided by slowing the ramping rate as the desired temperature is approached, but this extends the length of time required to complete the experiment and, in some situations, going more slowly can promote the formation of unwanted primer–dimer products.[12] Individual manufacturers have differing approaches for combating these factors, but ultimately, the physics of the block temperature regulation, as described earlier, limits the speed at which a stable and uniform temperature can be achieved.

The active temperature control system employed by the xxpress thermal cycler means that even while delivering a 10°C/s temperature ramp rate, it can control the under/over shoot to <0.5°C for <0.5 s. This compares very favorably with a typical performance of Peltier system that can cause an under/over shoot of typically ±4°C for >10 s when delivering only a 4°C/s ramp rate. This performance is a major factor in improving the consistency of the PCR experiment and thus in improving the validity of the results.

10.9 CAN CHEMISTRIES MATCH THE THERMAL CYCLER SPEED?

The xxpress thermal cycler can deliver very rapid and extremely accurate thermal cycling. Thermal ramp rates of 10°C/s save significant time over the current PCR profiles; however, that is not the

only component of reaction times. The dwell times at each of the temperatures in the cycle are also a major factor in the length of the entire reaction. Samples are required to dwell at a temperature for a particular reaction to occur; the faster these reactions occur, the shorter the required dwell time and the quicker the cycle.

The dwell time consists of two elements: First, a defined period of time is required to ensure that all the samples are at the required temperature. This time period, the stabilization time, is dependent on the PCR instrument and the chosen ramp profile and not on the reagents. As discussed previously, instruments overshoot and undershoot the desired temperature targets and it can be a number of seconds before the whole plate is within the required temperature window. The second element, the reaction time, is the actual time taken to complete that reaction step. The reaction time is determined by the reagents and the specific experiment design.

Reagent suppliers have made vast improvements in recent years, creating new products that complete the reaction steps quickly, with dwell times of 1 or 2 s being possible, once the reactions are at the correct temperatures.

Figure 10.5 shows a comparison between a traditional thermal cycler and the active control adopted by the xxpress system. The time for each phase of the cycle is reduced when using the xxpress system, leading to dramatic reductions in the length of the experiment. It is clear that it is the combination of the fast ramp rates, good thermal uniformity, and the short stabilization times that enable the latest reagents to perform to their best and deliver a 40-cycle PCR test in less than 10 min. However, even with slower reagents, significant gains can still be achieved by utilizing faster ramping and reduced stabilization times.

10.10 A USER INTERFACE THAT DOES NOT REQUIRE A MANUAL

As has been stated, PCR is the accepted gold standard test for researching DNA and the thermal cycler is the tool that enables that process; however, it should not be a barrier to it. In the past, many thermal cyclers required specialist training to be completed or long user manuals to be read before they can be used. So, in conceiving the xxpress thermal cycler, a key design goal for BJS was that the user, provided they understood the PCR process, should be able to operate their thermal cycler without having to refer to a manual or go on a training course. It was to meet the requirements of this goal that the xxpress touch screen user interface (UI) was created, a UI that would guide them through the process.

The UI on the xxpress thermal cycler is in the form of a "film strip" that moves from right to left across the screen. In the frames of this film strip, the user is asked to make key choices as to what they wish to do or input the specific experimental information. Much of the other information is prepopulated from data files on the reagents chosen. (Many processes for a user of a thermal cycler are repetitive; remembering the user's preferences and past setups make these tasks quicker and less irritating as well as leading to fewer mistakes.)

The process of experimental setup usually consists of just three steps: In the first step, the user identifies the type of qPCR they wish to perform and the reagents that they plan to use; this information is used to create the thermal profile and fluorescence measurement parameters automatically. These values can all be changed if required but they are based upon what the manufacturer of the reagent recommends. The second decision is the choice of the sample size and the number of tests; there are currently three plate formats for the unit: 24, 54, and 96 wells. Interchangeable, each of these fit into the unit without further modification. The unit automatically recognizes the format of the test plate that is put in the machine, makes any necessary adjustments, and checks to see if it matches what the user has requested. Once the test plate format has been chosen, the arrangement of the experiment can be planned. Genes of interest, reference genes, and no-template controls can all be added from the menu. Arrangements are always remembered so that future experiments can use the same layout. Finally, the thermal profile and measurement information is displayed so that any adjustments can be made before starting the program.

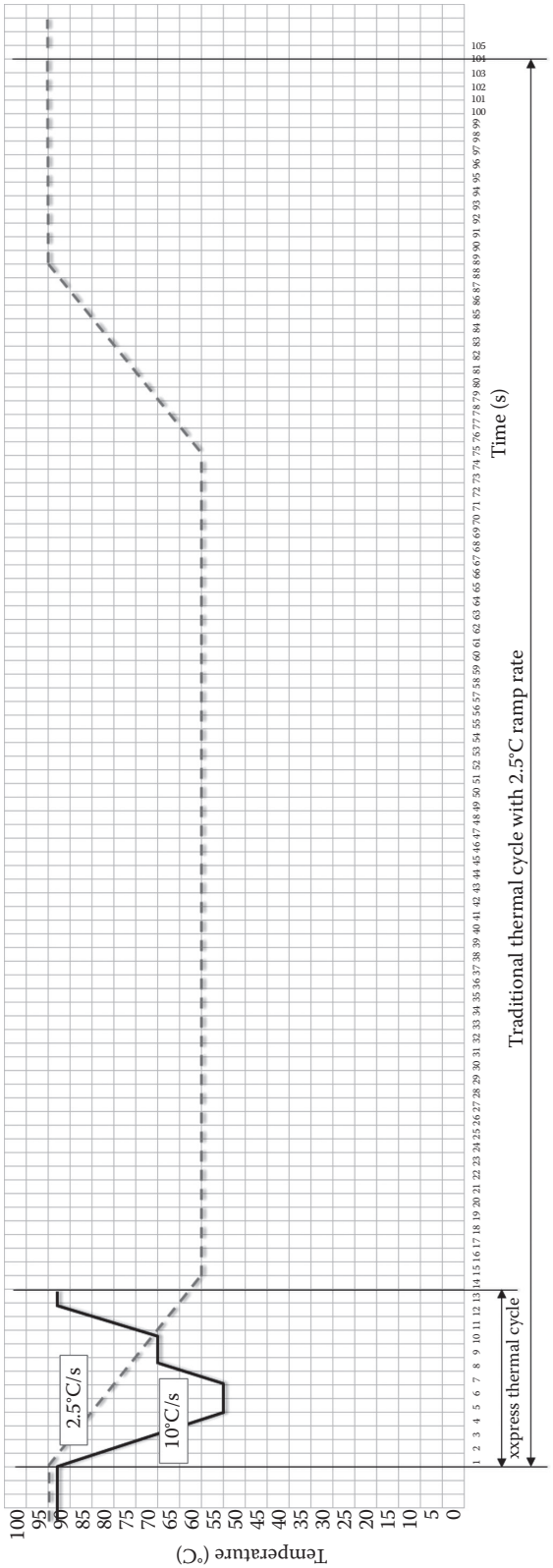

FIGURE 10.5 Comparative timing curves for traditional and xxpress PCR systems.

A similar approach is used to provide analysis of the data once the experiment is complete. This analysis as well as program creation can be done away from the machine to maximize the utilization of the instrument.

10.11 ADDITIONAL DESIGN CHALLENGES

When designing a new instrument, it is important not just to focus on a single strong-performance criterion but also to try to match that higher standard across all the features of the instrument, by delivering improvements in all the areas of performance. This is particularly true when a significant improvement in one feature can highlight a disproportionate limitation in another. The speed of the test process is an important metric, and so too is a total reaction cost. A crucial component of the cost of a PCR test is the reagent. Reagent costs are regularly 20 times the cost of the consumable test plate; minimizing that cost can provide significant savings. Typically reducing reagent costs requires adopting smaller test sample volumes while achieving the same results as in larger volume reactions. To address this challenge, BJS created a range of test plates that could handle different experimental volumes, ranging from 0.5 up to 40 μL using test plates that have a common format and are interchangeable (Figure 10.6). This change in design philosophy over the traditional systems enables an instrument to be used seamlessly by a number of users in a laboratory without creating delays or the potentials for error if the block format requires reconfiguration for each particular experiment.

10.12 BENEFITS OF ULTRA-HIGH-SPEED PCR

PCR has been improving continually ever since its inception approximately 30 years ago and as with all developments, some changes are small and often incremental whereas others occur through larger "breakthrough" improvements that can lead to more radical changes in the way a solution can be applied. Such is the case with ultra-high-speed PCR and the 10-min test; this technology will be disruptive to the accepted convention in that it will cause users to fundamentally review how they use PCR. Until relatively recently, the view of diagnostic testing was that it would be done centrally, in specialist laboratories using larger and evermore expensive equipment. The issues around timescales, the transporting of samples, managing of data, and the need to have patients visit their health care center multiple times, were managed. It was not regarded as inefficient as the alternative would have been not having access to these services at all. However, if these services can be provided closer to the patient on a "while you wait" basis, there will be many benefits to both the patient and the community at large. Access to the latest diagnostic testing could also be made available in less developed areas of the world, in countries where transport infrastructures and sophisticated centralized laboratories, a prerequisite to current solutions, do not exist.

A successful diagnostic test needs to fulfill two key criteria: It needs to be sensitive enough to detect the change that identifies the parameter being tested and it needs to be specific enough to determine that it is that change, and not another, which is the cause. This testing principle applies across all science but is most pertinent in the development of diagnostic tests. Every diagnostic test is assessed for its specificity and sensitivity. Our greater understanding of genetics has been the catalyst for recent breakthroughs in the design of new diagnostic tests; the identification of a genetic marker, DNA, RNA, or otherwise, provides a very specific and hence reliable test. The PCR process lies at the core of these developments because it is highly specific and sensitive, being able to detect as little as a single DNA strand. So, for a number of years, the focus of much of the work on the development of diagnostic tests has been in identifying genetic markers for key diseases and conditions.

An example of the impact of this work is in the area of human immunodeficiency virus (HIV) testing. Until recently, HIV was identified by testing for antibodies that are produced by the body to fight the infection. However, these antibodies can take over 12 weeks to develop, and sometimes as long as 12 months; so, providing a period of uncertainty where the patient may go untreated potentially becomes

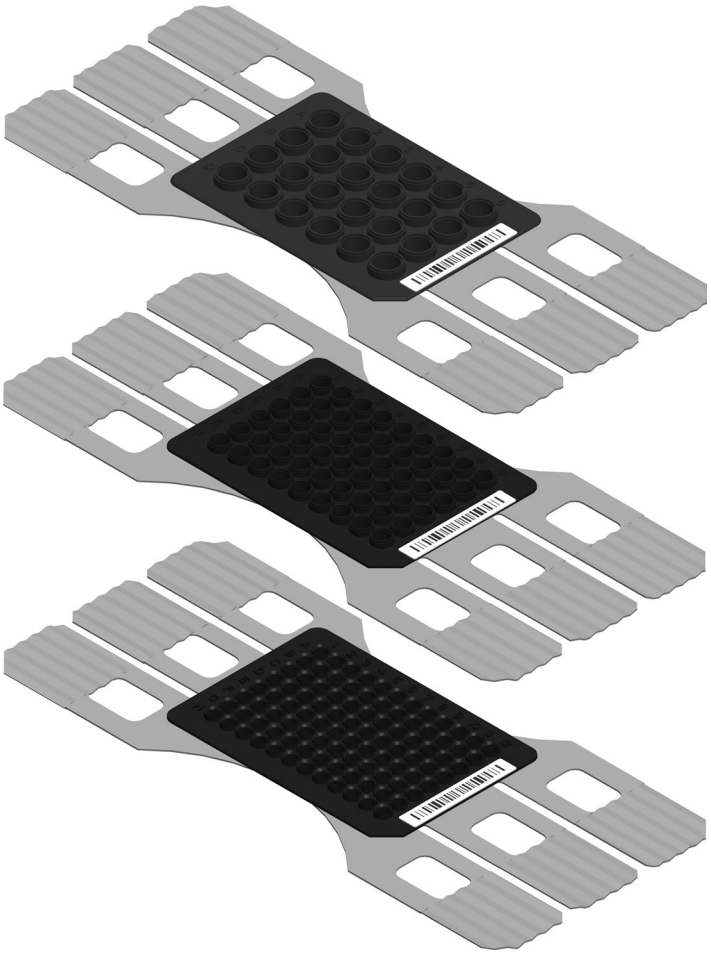

FIGURE 10.6 Three types of xxpress consumable test plates.

a source of infection to others. Recently, a reverse transcription (RT)–PCR-based test was developed that can detect one or more of the several target sequences located in specific HIV genes, such as HIV-I GAG, HIV-II GAG, HIV-env, or HIV-pol. This test enables testing within 3 weeks of possible infection. Such a major improvement enables earlier treatment and reduces significantly the stress from outcome uncertainty; this test is also now routinely used to screen blood supplies.[13]

While PCR-based tests have proved their effectiveness, these types of tests have the potential to provide significantly greater benefits if they could be provided locally where the patient interacts with the health care system so that the patient can be diagnosed and treated at the first visit.[14] One of the barriers to delivering these types of tests closer to their "POC" has been the time taken to get the results. If the results are not going to be available for a number of hours, then is it practical or useful to wait for them? In many cases, it is not. While if the test was to give results in around 20 min, then POC diagnosis becomes a possibility. There are many other elements to delivering fast tests at the point of need, such as the sample preparation that can take many hours and requires specialist laboratory techniques, but the focus on improving these other elements will become much sharper once the current time-limiting step, the PCR, has been redefined. It is not just in the field of medical diagnostics that a time saving in PCR test time could deliver both benefits and drive further development. There are a number of other

applications where this level of improvement in the PCR test time will create major revisions in how it can be used.

In food testing, the current PCR testing is done on a batch basis, with items often being shipped out of the production facility prior to the results being available. This can lead to public embarrassment and significant recall costs if a process fails. Clearly, on site, real-time testing providing continuous monitoring of the process, catching issues before shipment, and reducing potential waste would be a huge improvement over the current testing protocols.

Control and monitoring of epidemic outbreaks, both in humans and in animals, relies on the rapid turnaround of sample analysis and results to identify and isolate an outbreak. Examples of such critical situations were the U.K. foot-and-mouth epidemics of 2001 and 2007 where tens of thousands of animals were destroyed and their travel was severely restricted.[15] Quicker identification of the disease in flocks would have isolated the cases earlier reducing the impact to the livestock and the country. PCR test speed improvements can provide clear benefits in time-critical applications.

10.13 SUMMARY AND FUTURE STEPS

The change in philosophy from passive heating to active heating enables a system to be designed with very low thermal mass enabling both fast ramping and accurate temperature control across the whole sample area. The development of the technology for measuring and controlling an active heating system is challenging, but once completed, the benefits are significant. The resulting step change in cycle times for the PCR not only will enable general time savings to be made but could also facilitate the quicker delivery of many tests such that they could be provided in a different manner to how they are currently, on a "while you wait" or "in process" basis.

Looking ahead, the active heating technology is scalable and so can be applied to smaller individual test systems or larger automated batch-based platforms. This process is also significantly more thermally efficient than the traditional systems as the amount of energy being moved in and out of the sample is much less due to the lower thermal masses. What remains to be seen is the level of impact that this increased speed will have within the arena of PCR and against the other technologies available in the wider world of diagnostic testing.

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