

---

**PART III - DISCUSSION OF RESULTS AND CONCLUSIONS**

---

---

## 6. DISCUSSION OF RESULTS

---

### 6.1. FIELD OVERVIEW AND SIGNIFICANCE OF RESULTS

Conducting a well-documented discussion of the results here reported is not an easy task. Even without taking into account the vast nature of the work here undertaken, which, broadly, covers the whole range of experimentation conducted to date on PCR-chips, the task of elaborating a refereed discussion remains troublesome due to, precisely, the scarcity of cross-refereed material and, in general, a lack of intrinsic detail in reports concerning the development, optimization and use of PCR-chips. The obscure nature of the PCR-chips literature is not a special trait of this sub-field, but a broader problem facing the whole field of DNA-chips and stirred, mainly, by the economic prospects [Stipp1997] these systems brought forth in the early 1990's (a notable exception to this trend, mostly due to historical reasons, is the DNA-electrophoresis chips field). As a consequence, and as in other fields of seemingly economic interest (e.g. GM technology), the global picture of PCR-chips is, at the most, sketchy and crammed with poorly documented results that have almost never been reproduced (and consequently neither compared) by independent research groups. As a consequence, crucial issues (like insertion/extraction techniques, adsorption phenomena or the problem of providing airtight, but removable, sealing) have been overtly and unworriedly disregarded by almost all the research groups working on PCR-chips, even though, when addressed (as in the case of materials biocompatibility and PCR-mix optimization, see [Northrup1993], [Shoffner1996], [Taylor1997]), they have brought forth conflicting results. A remarkable instance of the murkiness engulfing PCR-chip literature can be already glimpsed in the field-foundational paper by Northrup *et al.*: "*It was found that none of the silicon-based materials had a significant inhibitory effect on the PCR; however, chemical silanization of the reaction chambers surfaces was carried out as a preventive measure.*" [Northrup1993]. On top of it all, the multifaceted nature of PCR and PCR-chips, and the very different technological and methodological approaches taken by different research groups (different substrates, heating elements, etc.), have all contributed to shed more confusion than light onto this field; a fact that has only been occasionally and scantily contravened by some research articles ([Shoffner1996], [Cheng1996a], [Zhan2000]). Therefore, and in the wake of this general cloudiness, the significance of the work here reported becomes two-fold: not only have new technological and

methodological techniques been introduced and extensively tested (together with fundamental issues in PCR-chip technology, such as biocompatibility, insertion/extraction methods or PCR-mix optimization), but a comprehensive survey of different reported methodologies has been carried out, establishing a solid link and providing the means for a first field-comparison between previously documented, but otherwise mainly unrelated, approaches.

---

## **6.2. COMPARISON OF DRIVING AND SENSING MECHANISMS**

As mentioned above, one of the main imports of this work has been the simultaneous development and comparison of different technological and methodological strategies. Among these, perhaps one of the deepest dividing lines between different research works has been the split into integrated and external heat driving (and consequently sensing) mechanisms.

### **6.2.1. DRIVING MECHANISMS**

Due to the own nature of PCR, heat-driving mechanisms are an essential part of PCR-chips. Although the introductory work on PCR-chips did already bring forth integrated heaters/sensors [Northrup1993], the main basic research work ([Shoffner1996], [Taylor1997]) on these systems has been carried out using external Peltier-driven systems and sensors, and has assumed that there were no intrinsic differences between active and passive PCR-chips. Hence, even though, intuitively (and provided that heat/cooling rates abide in the same ranges for both systems) this is not a naïve assumption, there has been no attempt at establishing a common ground for comparison between these systems. The functional results here reported for passive (see p.202-212) and active (see p.244-247, 255-258) PCR-chips confirm the hypothesis that there is no intrinsic difference between both kinds of systems; that is, no intrinsic difference whenever transient behaviors are equivalent. In fact, quantitative analyses of PCR results within this work (data not shown) hint at a slightly superior PCR efficiency in passive PCR-chips. The most probable explanation for this fact, discarding improper sensor calibration, is that transient behaviors and, most importantly, inertial and sensor-triggered overshoots were not equal for both kinds of systems. Peltier-driven systems portrayed poorer transient rates and both inertial and sensor-triggered overshoots (mostly during cooling) that could readily explain the observed better efficiency. Nonetheless, even when

identical transitional properties for both systems are assumed, the assumption does not come for free. As discussed previously (see p.230), integrated heating elements provide much more power-efficient cycling than Peltier cells in the centimeter-chip range, since they do not introduce a significant external mass. Exactly in which size-ranges does this hold true is not a trivial question (specially if the better cooling-rates of Peltier cells are taken into account) and is a matter open for investigation.

### **6.2.2. SENSING MECHANISMS**

Even though composite systems (like that reported in p.245) can be readily implemented, the general rule of thumb in PCR-chips has been to assume that integrated heaters come with integrated sensors and vice versa. Therefore, a comparison between external and integrated heat-driven systems is, by definition, a comparison between external and integrated sensors. In the light of this, the composite system (integrated actuator plus external Pt100 sensor) results here reported are of singular significance, since they envisage an intermediate boundary between both classical kinds of systems. In agreement with previously conjectured hypotheses, experimental results for the composite system depict mixed outcomes. As expected, total yields are a bit lower than those obtained with Peltier-driven systems, but still larger than those of independent-control active chips, due to the somewhat larger stabilization times required by the external sensor. At the same time, PCR specificity is handicapped by a lower cooling rate (lower than in independent active PCR-chips) and sensor-triggered overshoots during cooling. This is markedly indicated by the presence of secondary amplicate structures that, following the results of *Wittmer et al.* on capillary PCR ([Wittmer1989], [Wittmer1990]), aptly disappear in independent active PCR-chip operation (see p.256).

---

### **6.3. DESIGNS COMPARISON**

At the microscopic scale of chip PCR, design is a fundamental issue with compelling repercussions on a variety of chip functional properties (e.g. tensional forces that influence on insertion and extraction performance or adsorption phenomena that have strong effects on efficiency). Therefore, it is surprising to find out that no systematic design studies have been conducted (or at least reported) up to very recently and that, when conducted [Lin2000a], they have only shed light on some of the involved

issues. The results here reported yield more than telltale signs of the influence of design parameters in insertion/extraction procedures and, a priori, cast some doubts on the validity of research carried out by some groups. For instance, *Shoffner's* team designs ([Shoffner1996], [Cheng1996a], [Cheng1996b]) have been reproduced and found to pose extremely difficult problems in insertion and extraction procedures and yields (see p.131), a fact that should be more acute in *Shoffner's* group implementation, since their less profound (80  $\mu\text{m}$  deep in contrast to the here-reported 125-150  $\mu\text{m}$  deep) reservoirs should accrue the surface-tensional effects that hamper insertion and extraction of reagents. Similarly, *Taylor's* team back-opening designs [Taylor1997] have also been reproduced here (see p.131) and have been shown to yield similar insertion/extraction problems, while generating extremely fragile chips that often broke during handling. Both issues may be partly contravened in *Taylor's* chips due to their use of thicker (0.7 mm) wafers. This allows them to create deeper reservoirs (0.5 mm deep) and, consequently, slightly smaller planar dimensions and thicker membranes. Even so, their reported relative sizes (17.3.5 mm<sup>2</sup> chips with 200  $\mu\text{m}$  membranes) do not lie excessively far from those implemented here, thus casting some doubts on the validity of their approach.

Finally, a classical [Kopp1998] and obvious alternative in PCR-chip design (square versus serpentine-like chips) is discussed by *Lin et al.* [Lin2000a] in terms of its influence on insertion and, mostly, extraction efficiency. In their paper, *Lin et al.* report that only 5  $\mu\text{l}$  out of their 50  $\mu\text{l}$  sample could be expelled from rectangular reservoirs (a result consistent with those obtained here for similar designs, see p.131), and conclude that switching to serpentine-like chips (displaying 80% sample recovery) is the best design option for PCR-chips. Nevertheless, in their assay, *Lin et al.* fail to address theoretically problematic issues (like a decrease of adsorption phenomena in serpentine-like chips due to their decreased surface-to-volume ratio or a possible lesser efficiency due to poorer reaction kinetics) through a comparative assay with rectangular chips, nor do they offer any alternative design ideas to circumvent the extraction problems of rectangular chips. Both these issues have been largely addressed here. The basic rectangular designs of *Shoffner* and *Taylor* teams have been evolved and optimized into the rhomboidal chips here described (see p.143) and, with the introduction of the appropriate external machinery (see p. 145), insertion/extraction yields have been raised to 95% and 90% in serpentine-like and rhomboidal chips (see p. 145). Accordingly, quantitative PCR yields have been

repeatedly compared for both designs (see p.247, p.258) and it has been demonstrated that a switch between these shapes does not influence chip PCR efficiency in a substantial manner.

---

#### **6.4. SURFACE BIOCOMPATIBILITY**

Surface biocompatibility is, obviously, a major issue in PCR-chip fabrication, since it can preclude chip operation and has important effects on chip mass-production schemes. Hence, although this issue has been addressed twice in the literature ([Northrup1993], [Shoffner1996]), it is startling that their conflicting results have not been addressed later. In essence, *Shoffner's* article undermines *Northrup's* confusing remarks on materials biocompatibility (see p.263) and states that some silicon-related materials do indeed show signs of PCR inhibition (such as bare silicon and silicon nitride), whilst surface silanization techniques are deemed non-determinant and too unreliable for mass production. The vast majority of the following research has relied on *Shoffner's* assumption that silicon oxide is the best biocompatible layer for PCR-chip production and the issue has remained unchecked to date. Although lacking some methodological rigor, the biocompatibility results obtained here for different passivation layers are in accordance with those reported by *Shoffner*, even though the main conclusion from the powder and fragment PCR-inhibition assays here conducted is a bit different from that of *Shoffner's* work. The documented results indicate that silicon (nor polysilicon) is not a direct inhibitor of PCR (a material that would cause inhibition even if present in very small proportions), but that the silicon-inhibition effect seen in these assays is, at heart, an accrued case of the adsorption-inhibition effect seen in silicon dioxide surfaces, and is mostly due to the increased surface-to-volume ratio of PCR-chips.

---

#### **6.5. PCR-MIX OPTIMIZATION AND ADSORPTION PHENOMENA**

The notion of surface interaction phenomena in PCR-chips was first introduced by *Shoffner et al.* [Shoffner1996], but its first definition as adsorption phenomena is found in *Taylor's* 1997 paper [Taylor1997]. *Taylor et al.*, in addition, were the first to suggest the addition of BSA as a carrier protein in the PCR-mix that would compete with Taq polymerase for adsorption at the chip walls and thus improve PCR yields. Even though many further experiments have involved the addition of BSA into the PCR-

mix ([Waters1998a], [Lin2000a], [Zhan2000], [Murakami2000]), titration experiments and quantitative effects of this addition have not been reported, and BSA addition correlation with either Taq or DNA adsorption has not been thoroughly addressed. In the present research, the effect of BSA addition into the PCR-mix has been methodically addressed, showing that BSA does indeed compete for adsorption with Taq polymerase and that DNA adsorption is not a relevant issue in PCR-chips (see p.194). Moreover, BSA concentrations have been extensively and repeatedly titrated in different chip setups (see p.200, p.258), indicating that a convenient BSA concentration must lie in the whereabouts of 2-3  $\mu\text{g}/\mu\text{l}$ .

---

## **6.6. INSERTION/EXTRACTION AND SEALING OPTIMIZATION**

For years, the nature and methods of the  $\mu$ -TAS interface with the external, macroscopic world have remained a largely unaddressed issue, with researches focusing mainly, and naturally, on the demonstration of specific  $\mu$ -TAS capabilities. However, in recent years, and as the bottleneck nature of such matters has started to be assessed, this trend has subtly changed, even though much research is still required to assess the multifaceted problems of  $\mu$ -TAS interfaces (see p.49). PCR-chips are not an exception to this global tendency and, consequently, it comes as no surprise that very little research in this particular has been published. In the case of PCR-chips, the problem is aggravated by the own nature of these systems, which require, on the one hand, airtight sealing during operation and, on the other hand, easy insertion/extraction methods. This imposes certain restrictions on the use of, for example, glued tubes (which are of difficult sealing) and casts many clouds on the feasibility and functionality of many designs (see p.100). Moreover, the nature of insertion/extraction and sealing procedures has also repercussions on washing, sterilization and reuse issues, which have also been overly disregarded in PCR-chips research. In this sense, even though sterilization might not be necessary in mass-produced, disposable PCR-chips, this is not a definitive assertion (since sterile conditions such be imposed at clean room facilities and sterile packaging would be required for delivery, driving up costs). Therefore, the development of techniques for efficient, sterilization-compatible and reuse insertion/extraction and sealing are seen here as a major commitment in PCR-chip research.

Since the early work of *Northrup* [Northrup1993], in which silicone and polyethylene tubing were used to accomplish reagent insertion/extraction and device sealing, the main body of research work has relied in the use of acrylic tape sealants and direct chip pipetting for the same purposes ([Taylor1997],[Lin2000a],[Lin2000b], [Zhan2000]). A notable exception to this trend has been *Wilding and Shoffner's* use of small sealing washers ([Wilding1994], [Shoffner1996]) and the introduction of mineral oil as the sealing agent by Burns et al. [Burns1996]. In contrast, airtight devices have been commonly accomplished by means of anodic bonding of a glass cover onto the silicon chip. In the present work, different sealing and insertion/extraction devices have been investigated and optimized to achieve efficient insertion/extraction rates while maintaining compatibility with washing and sterilization protocols. Even though other technological methods have been approached (see p.115), anodic bonding to glass wafers has been preserved here, since it does not introduce any extraneous materials, it offers effective and resistant bonding and maintains compatibility with other application processes (s. c. electrophoresis chips). Concerning insertion and extraction procedures, the development and optimization of independently washable insertion/extraction methacrylate devices here undertaken (see p.128, p.145) provides the means for, in conjunction with chip designs (see p.142), very effective insertion and extraction yields (up to 99% in serpentine-like chips). Similarly, the development of alternatives to washer and acrylic tape sealing in passive PCR-chips and the creation of dual toric-joint sealing & electrical access Teflon devices for active PCR-chips has resulted in very effective and reproducible, evaporation-free chip sealing, while hinting at serious inconveniences of previous approaches. For instance, supposedly biocompatible acrylic tapes as those used by *Taylor et al.* [Taylor1997] have been shown to seriously inhibit PCR (see p.177) and, although this problem can often be overlooked if air is (as it typically is) present at chip reservoir ends, it is nonetheless a serious drawback that can preclude reproducibility in these sealing schemes. Moreover, acrylic tape sealing has been found to create some chip breakage problems during manipulation (see p.180), and it is clearly an unreliable technique. Similarly, the problems posed by the use of washer sealing (inefficient contact with the heating-cooling surface) in back-etched PCR-chips have been studied here and found to be of very difficult modeling (see p.181). In this sense, the design of replica sealing chips (see p.185) has proved an enlightening option for effective sealing of back-etched passive PCR-chips, without the addition of excessive thermal mass layers or extraneous



materials (like mineral oil) of less reliable sealing-effectiveness (see p.182). Likewise, the development of electric-contact Teflon sealing mechanisms has provided the means for simple, reproducible and effective airtight sealing and electrical access without complicating chip design, nor introducing foreign or glued elements.

All in all, the set of methods and techniques here described for chip sealing and reagent insertion/extraction offer the highest insertion/extraction yields reported to date, while providing simple operation and fabrication, together with independent washing procedures that guarantee chip sterilization and reuse. Due to this, the techniques here described are of easy scaling up, both in terms of replication and automation (for example, in multi-chip holder modules), and in terms of batch manufacturing.

---

## **6.7. DEVELOPMENT AND CHARACTERIZATION OF A CMOS COMPATIBLE PROCESS FOR ACTIVE PCR-CHIPS**

The technological integration of PCR-chip fabrication processes in a CMOS compatible setup is of fundamental interest to the outcome of this kind of devices. As *Shoffner* points out when considering the advantages of silicon dioxide over silanization procedures [Shoffner1996]: "*The discovery of an inert, 'PCR friendly' [and process integrated] surface for microfabricated silicon devices is one important step towards the construction of an integrated, inexpensive, automated microfabricated PCR analysis system.*" Likewise, the introduction of anodic bonding as a standard, reproducible sealing technique is another step towards this goal, posing strong advantages over silicone and other glue-like bonding mechanisms. Nonetheless, since the early work of *Northrup* [Northrup1993], which did not include anodic bonding, no CMOS easily adaptable processes have been described for active PCR-chips. This is partly due to the already mentioned convergence of research around passive PCR-chips ([Wilding1994], [Shoffner1996], [Taylor1997], [Lin2000a]), and to the fact that the only reported active PCR-chip processes ([Poser1997], [Zhan2000]) have not risked the seemingly unreliable accuracy ( $\pm 1.5$  °C) of polysilicon resistors reported by *Northrup* and, therefore, have relied on the more standard thin-film platinum resistors for temperature sensing.

Although thin-film platinum deposition is becoming an extended practice in most microsystem clean-room facilities, it is still not a standardized process and its inclusion can unintentionally, but handsomely, complicate the

global fabrication process of PCR-chips. Moreover, typical platinum resistance values make it hard to design large sensing resistors motifs that can provide accurate mean temperature distribution across the PCR reservoir (a problem keenly observed by *Zhan et al.* [Zhan2000]). Hence, the technological integration and design adaptation conducted here for the achievement of a simple, single-layer technological process for active PCR-chip production suits a handful of goals. On the one hand, a simple CMOS-compatible technological process has been proposed, implemented and systematized for the full batch-like production of active, sealed and PCR-friendly passivated PCR-chips. On the other hand, and in the process of doing so, a feasible design alternative for sensor and actuator grids has been brought forth and its reliability has been tested against accuracy (a nominal  $\pm 0.25$  °C deviation, see p.250) and common sensing problems such as those reported by *Zhan et al.* The result is a combination of both design and technological process innovations that are capable of delivering complete, active PCR-chips using a relatively simple CMOS-compatible technological process and functionally tested design approaches.

---

## 6.8. SYSTEM PERFORMANCE

In addition to the aforementioned points, the results here reported demonstrate the functionality and reproducibility of the developed PCR-chips over a wide range of templates and cycling conditions. Moreover, whilst the yields obtained with these devices are similar to those published to date ([Northrup1993], [Shoffner1996], [Taylor1997]), the concentration of essential reagents has been scaled down and optimized with respect to the same reports. In particular, Taq polymerase concentration has been titrated in conjunction with BSA, and yields similar to those of thermocycler positive controls have been accomplished with very low Taq polymerase concentrations (0.05 U/ $\mu$ l), while reproducible positive results have been obtained for even lower concentrations (0.028 U/ $\mu$ l). Primer and dNTP concentrations have also been scaled down, while the chip PCR sensitivity has been driven down to a mere 1.2 ng/ $\mu$ l of template DNA.

---

## 6.9. LOOSE ENDS AND FURTHER EXTENSIONS

Albeit much work has been done on the development of PCR-chips, many loose ends and possible work lines remain open for further research and development. Nevertheless, the work here reported has the import of

comprising an extensive insight into the world of PCR-chips and of having built a knowledgeable ground base from which further extensions may be attempted with a firm grip on technological and design issues.

### **6.9.1. LOOSE ENDS AND BASIC EXTENSIONS**

Among the different developments that can be incorporated into the present work, one of the foremost comprises the optimization and extensive testing of the devices here reported. In this sense, once feasible and reproducible reactions have been demonstrated, careful titrations should be carried out for every PCR-mix component and new adjuvants (like DMSO or betaine), with the aim of improving the yields of the here developed PCR-chips. Similarly, technique modifications (such as hotstart or touchdown PCR) and different passivation layers (i.e. thicker and thinner, wet and dry SiO<sub>2</sub> layers, and polysilicon and nitride layers) should be functionally studied with identical aims, and some silanization procedures ought to be developed and crosschecked with these results. In addition, the foretold advantages of PCR-chip operation predicted by *Wittmer* ([Wittmer1989], [Wittmer1990]; better specificity and efficiency), together with improvements in cycling speed (zero hold times and the like), which have only partially been addressed here, should be analyzed in depth and contrasted with the yields provided by similar devices, such as commercial capillary-based thermocyclers (e.g. the *Lightcycler*, [Erill2002]).

### **6.9.2. FURTHER EXTENSIONS**

A welcome, even if lengthier, extension to the study here depicted should be a progressive analysis of the feasibility and ensuing problems and advantages of scaling down the PCR-chips here developed. On the basis of the empirical results here reported and of theoretical approximations to the behavior of PCR, it can be forecasted that a scaling down of PCR devices should present some inconveniences, such as problems arising from the prevalence of fluid tensional forces, but should also bring some interesting advantages. Disregarding the use of smaller volumes of reagents, downscaling would also convey faster and more accurate cycling, a lesser incidence of surface adsorption phenomena (due to a decreased surface-to-volume ratio), as well as the ultimate test bench for a sound comparison between Peltier cells (which at smaller scales could even become efficient enough for integration) and thin-film heaters.

Finally, integrating other analytical functions into the here developed PCR-chips would be the dream of every  $\mu$ -TAS researcher. Nonetheless, and even though some nice additions (like the coupling of electrodes for *in-situ* cell lysis and the addition of passive filtering schemes [Wilding1998]) are not far-off dreams, the experience gained from the work here reported teaches a somewhat different lesson. It seems obvious that, in a near future, the ultimate  $\mu$ -TAS device will display as its prime feature the complete integration of a complicated series of analytical functions, but, even so, there is no straightest path towards this goal. From the standpoint of this research, it can be seen that aiming at an advancing integration of analytical functions may not be the best short/mid term objective for PCR-chips in the scientific (nor in the commercial) arena. Instead, a closer and more feasible market niche can be sought by the integration of PCR-related functional capabilities on the chip (e.g. integrated detection) and the specialization of these devices in functional roles that are not attainable by other methods. For instance, multi-chip programmable modules (which allow independent cycle programming) and portable, battery-powered PCR instruments [Northrup1995] would be welcome product innovations based on the exclusive properties of PCR-chips. Likewise, closing the bridge (with the addition of functional capabilities) to a full, even if PCR-limited,  $\mu$ -TAS would be a more realistic approach towards these systems. In this sense, the easily adaptable compatibility of the here reported technological processes with standard CMOS batch fabrication and the reported use of independent external insertion/extraction and sealing machinery both pave the way for the pursuit of both the above-described trends. The possible combination with standard and other CMOS processes allows the integration of many functional capabilities (control circuitry, programmable memory, integrated detectors). Of these, the development and integration of optical or electrical quantitative detection methods would be of the utmost importance, since it would get rid of one of  $\mu$ -TAS deepest problems (the output interface with the macroscopic world), while bringing forth the possibility (due to their proximity) of reaching today unattainable detection resolution limits.

---

## 7. CONCLUSIONS

As an end to this dissertation, and in the light of all the work here reported and of the above-detailed discussion of results, the following main conclusions can be drawn:

1. A complete, CMOS-compatible technological process for the production of fully operational active and passive PCR-chips has been developed and systematized, in accordance with a wider-scope, common-ground, CMOS-adaptable generic process for DNA  $\mu$ -TAS.
2. New and innovative integrated sensor and actuator designs based on the use of a single thin-film polysilicon layer have been conceived and positively tested.
3. A full-fledged study and a systematic optimization of PCR-chip design parameters have been conducted, providing new insights into the correlation between design issues and insertion/extraction and yield efficiencies. In this sense, extraction yields have been raised to functional (>90%) efficiencies, whilst the first field-comparison between fundamentally different kinds of designs (rhomboidal vs. serpentine-like) has been carried out and found out to be mainly irrelevant in terms of PCR efficiency.
4. Similarly, a clear-cut assessment of active and passive PCR-chip approaches has also been conducted for the first time, and their inherent advantages and disadvantages in terms of yields, power consumption and operability have been methodically contrasted.
5. In addition, the phenomena of Taq and other reagents adsorption to chip walls has been seriously and methodically addressed here for the first time, and its correlation with PCR efficiency has been clearly demonstrated and successfully overcome with the addition of carefully titrated amounts of BSA, resulting in efficient chip PCR operation at low Taq and template DNA concentrations.
6. To accomplish the above-mentioned goals, and coupled with chip design optimization efforts, a complete custom-tailored set of mechanical devices for reagent insertion/extraction and chip sealing has been developed and optimized, providing the grounds for easy and highly efficient operation (>90% extraction yields, 100% airtight sealing) with

PCR-chips, and the required independence for the introduction and standardization of cleansing and sterilization procedures that allow for repeated chip reuse and astringent sterilization whenever these are required.

7. It can be summarized, therefore, that the goals set at the beginning of this doctoral research have been manifestly reached: a complete set of PCR-chip processes, designs and external machinery has been developed and extensively tested, and the thereby produced PCR-chips have been found to be capable of implementing efficient and reproducible PCR analyses in shorter times than those of standard PCR instrumentation.