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Rapid PCR for Identity Testing Using a Battery-Powered Miniature Thermal Cycler

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ABSTRACT: A microfabricated, battery-powered thermal cycler was implemented in PCR-based DNA typing for human identification. HLA DQα and an STR triplex were PCR amplified using a device known as the Miniature Analytical Thermal Cycling Instrument (MATCI). The extremely efficient heating properties of the MATCI enabled thermal cycling to be completed in as little as 21 min. In addition, the feasibility of using the real-time fluorescent detection system of the MATCI was demonstrated. The successful application of this portable, prototype device to forensic identity testing is a significant milestone towards the eventual development of a completely integrated DNA testing instrument that would also incorporate sample preparation and allele detection.

KEYWORDS: forensic science, rapid PCR, DNA typing, microchip

The development of an integrated DNA identity testing instrument for rapid sample analysis requires the application of microfabrication technologies with conventional molecular assays (for reviews, see Burke et al., 1997; Abramowitz, 1996). Microchip based devices have been demonstrated to perform various aspects of DNA analysis, including sample preparation (2), PCR (3–7), capillary electrophoresis (8,9) and hybridization to oligonucleotide probe arrays (10–12). More notably, process integration, such as PCR and capillary electrophoresis, has been reported (13). Recently, Lawrence Livermore National Laboratory (LLNL) engineered a miniature analytical thermal cycler instrument (MATCI) that is powered by 13 rechargeable NiCd batteries, and has real-time two-color fluorescent detection (14). The entire instrument, consisting of a thermal cycler, solid-state optics, and a laptop computer, fits in a medium-sized briefcase, which together weighs 35 pounds.

In this report, the MATCI was applied to human identity testing. PCR products were rapidly generated for HLA-DQα and an STR triplex. The successful results clearly demonstrated the important role that miniature analytical devices will have in forensics.

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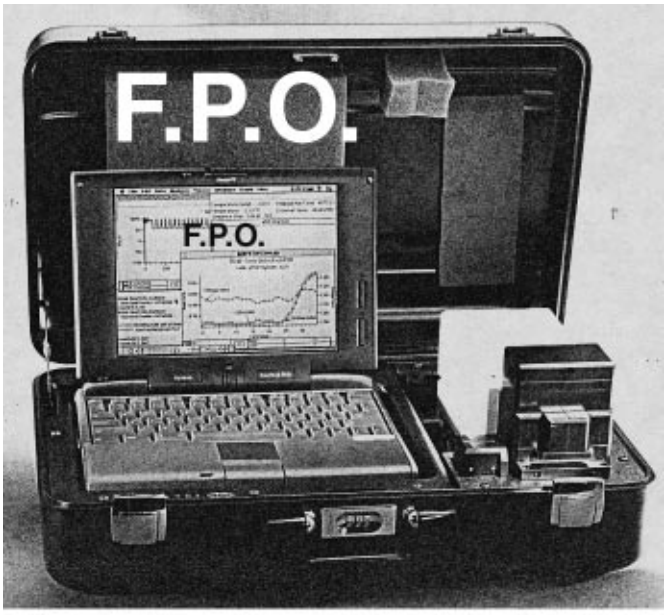
Materials and Methods

PCR Amplification and Typing—DNA was purified from whole blood using the Puragene DNA Isolation Kit (Gentra Systems, Mineapolis, MI). PCR amplification and typing for HLA DQα was performed using the commercial Amplitype HLA DQα (Applied Biosystems-Perkin Elmer, Foster City, CA) typing kit. PCR amplification of the AmpFISTR Blue STR system (Applied Biosystems-Perkin Elmer, Foster City, CA) was accomplished using either Mix 1 [AmpFISTR Blue PCR Reaction Mix, AmpFISTR Blue primers, 100 μg/mL BSA, 10 ng DNA, and 4 units AmpliTaq Gold DNA polymerase (Applied Biosystems-Perkin Elmer)] or Mix 2 [1X PCR II buffer (Applied Biosystems-Perkin Elmer), 3 mM $MgCl_2$, AmpFISTR Blue primers, 0.4 mM each dNTPs, 100 μg/mL BSA, 10 ng DNA, and 4 units AmpliTaq Gold DNA polymerase]. Thermal cycling was achieved with either the GeneAmp PCR System 9600 thermal cycler (Applied Biosystems-Perkin Elmer) or the miniature analytical thermal cycler instrument (MATCI) (LLNL, Livermore, CA).

The Miniature Analytical Thermal Cycler Instrument (MATCI)—The battery-powered MATCI was designed and assembled at the LLNL Microtechnology Center. The instrument (12 cm × 27 cm × 14 cm) contained a PCR microchip (0.6 cm × 2.2 cm × 0.2 cm), a solid-state optics system for real-time fluorescent detection, 13 NiCd batteries, and an electronic controller for computer interfacing (Fig. 1). Fabrication of the PCR microchip and other components have been previously described (3–5,7,13). Thermal cycling was controlled by a Macintosh Powerbook 5300C laptop computer using a program written in IgorPro (WaveMetrics, Lake Oswego, OR). The MATCI and the computer were fitted in a briefcase (33 cm × 54 cm × 18 cm). PCR was accomplished by placing ~25 μL of PCR mix in a disposable, custom thin-walled polypropylene sleeve that was designed and manufactured at the LLNL plastics shop. The mix was overlayed with ~3.5 μL of mineral oil. The sleeve was inserted into the opening at the top of the PCR microchip, and the thermal cycling program was initiated. When real-time fluorescent detection was performed (Fig. 2), the LED light source was inserted over the PCR microchip prior to thermal cycling.

Gel Electrophoresis and Fluorescent Detection—A 3 μL aliquot of AmpFISTR Blue PCR product (diluted 1:5) was mixed with 3 μL of formamide containing fluorescently labeled Genescan-2500



(a)

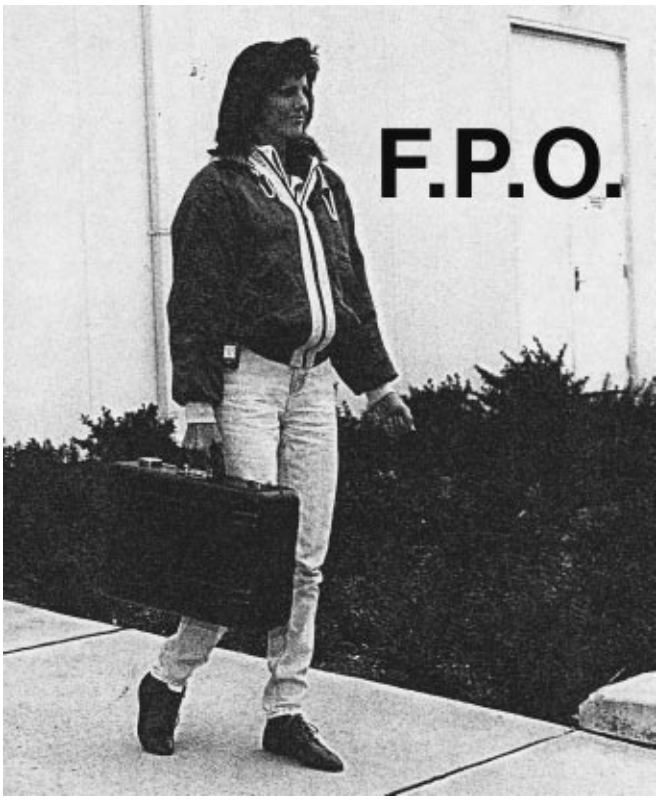


FIG. 1—*The Miniature Analytical Amplification System (MATCI). (A) The medium-sized briefcase contained a Macintosh PowerPC laptop computer and a microfabricated instrument, consisting of a PCR microchip, a solid-state optics system, 13 NiCd rechargeable batteries, and an electronic controller for computer interfacing. (B) The MATCI can be readily transported and operated at any location.*

[ROX] size standard (Applied Biosystems-Perkin Elmer). The sample was heated at 95°C for 2 min, quick cooled in ice, and electrophoresed through a denaturing 6% polyacrylamide gel in an Applied Biosystems 373 DNA sequencer.

Results and Discussion

HLA DQα typing using the MATCI (Fig. 1) and the GeneAmp PCR System 9600 thermal cycler (PE9600) was evaluated. A typical DQα thermal cycling procedure (94°C for 30 s; 30 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s; and 72°C for 10 min) was performed (Table 1, DQα, A and B). The reverse dot blot strips prepared from the products generated by each system were identical, with both strips plainly displaying the 1.2, 2 genotype (Fig. 3, compare A and B). The exceptional heating capabilities of the MATCI enabled PCR to be completed 25% faster than the PE9600 (Table 1, DQα, A and B). Ramping the temperatures from 60°C to 72°C and from 72°C to 94°C only required 2.9 and 2.7 s, respectively (Table 2). These rapid heating properties were further exploited by progressively decreasing the soak times to as little as 5 s at each temperature. Despite reducing the total time of PCR to only 21 min (Table 1, DQα, D), excellent typing results were observed (Fig. 3D).

The real-time detection system on the MATCI was assessed by spiking a DQα PCR mix with ethidium bromide, and subjecting the reaction to coupled thermal cycling and fluorescent analysis. The accumulation of DQα PCR product was monitored over the course of thermal cycling by utilizing the solid-state optical system built into the instrument. As ethidium bromide intercalated with PCR product generated during the reaction, the intensity of the fluorescent signal emitted by the ethidium bromide increased. The strength of the fluorescent signal (590 nm) detected by the photodiode was directly proportional to the amount of PCR product in the reaction (Fig. 2). The emission profile exhibited a typical sigmoidal detection curve.

Next, PCR amplification of the AmpFISTR Blue STR system was performed with the MATCI. AmpliTaq *Gold* DNA polymerase was substituted for the traditional AmpliTaq DNA polymerase. Preliminary work with STR systems had indicated that reaction conditions required modification to successfully achieve rapid multiplex PCR. Therefore, two reaction conditions were employed, the normal manufacturer's reaction mix (Mix 1) and a modified reaction mix (Mix 2). A series of fast thermal cycling profiles were utilized (Table 1), and PCR products were analyzed on an ABI 373 polyacrylamide slab gel electrophoresis system (Fig. 4). Based on the uniformity of peak height and prescence of abscence of peaks, Mix 2 (Fig. 4, bottom panels A-D) seemed to produce better overall results than Mix 1 (Fig. 4, top panels A-D). Mix 1 resulted in the loss of the FGA product in panel C (top) and virtually no detectable products in panel D (top). Mix 2 was more amenable to rapid PCR since all the products were still present in panel C (bottom) and two sets of products were observed in panel D (bottom). The results in panel C (bottom) were very encouraging, especially since total time of PCR was 60 min (Table 1C). When normal thermal cycling parameters (94°C for 11 min; 30 cycles at 94°C for 60 s, 60°C for 60 s, and 72°C for 60 s; and 72°C for 30 min) and the PE9600 were used, over 2.6 h was required to perform PCR. It should be noted that the actual PCR time for panel C, after subtracting the incubation at 94°C to activate the AmpliTaq *Gold* DNA polymerase and a final incubation at 72°C, was 42 min. Interestingly, the vWA alleles were still very robust in panel D

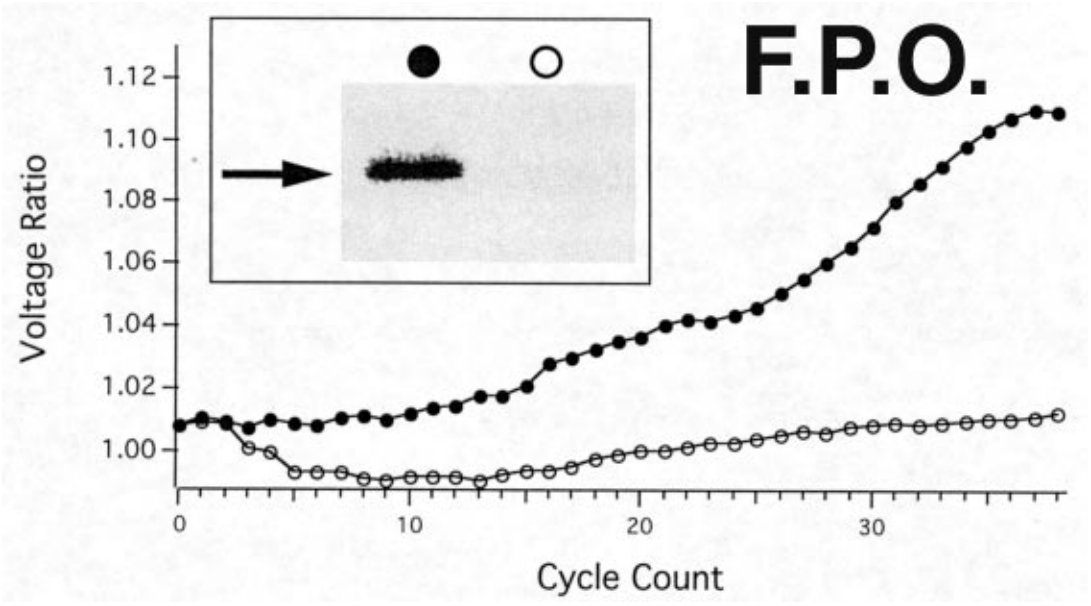


FIG. 2—*Real-time fluorescent detection of DQα PCR product. DQα PCR mix, containing either 10 ng of genomic DNA (closed circles) or no DNA (open circles), was spiked with ethidium bromide (3 μg/mL), and amplified using the MATCI. Ethidium bromide fluorescence was monitored using the solid-state optics system. An LED provided excitation light at 500 nm. Emitted light at 590 nm and 530 nm was collected and detected. The y-axis represents the ratio of signal at 590 nm to signal at 530 nm. Ethidium bromide has an emmission maximum at 605 nm, and should be primarily detected at 590 nm. Some background signal detected in the negative control (no DNA) was the result of trace primer-dimer formation. Reactions were run on a 2% agarose gel (left, upper box) to check for the presence of product. Arrow denotes the DQα PCR product. No product is detected in the negative control.*

TABLE 1—*PCR parameters.*

Test	Condition	Thermal Cycler	Soak Times (sec)	Final Extension Time (min)	Total Time* (hr:min:sec)
DQα	A	PE9600	30,30,30	10	1:39:32
	B	MATCI	30,30,30	10	1:14:47
	C	MATCI	10,10,10	10	39:30
	D	MATCI	5,5,5	1	21:18
STR	A	MATCI	10,45,30	7	1:17:25
	B	MATCI	10,30,30	7	1:09:56
	C	MATCI	10,30,15	7	60:28
	D	MATCI	10,10,10	7	45:44

*Includes an initial incubation at 95°C for 30 s for DQα and 11 min for the AmpFSTR Blue STR triplex. Soak times represent temperatures at 94°C, 60°C, and 72°C respectively.

TABLE 2—*Heating and cooling rates for HLA DQα and STR PCR.*

		PE9600		MATCI	
Ramp	ΔT(°C)	t(sec)	ΔT/t (°C/sec)	t(sec)	ΔT/t (°C/sec)
94 → 60	−34	18.1 ± 0.4	−1.9	15.6 ± 1.8	−2.2
60 → 72	12	21.9 ± 0.1	0.5	2.9 ± 0.1	4.1
72 → 94	22	33.2 ± 0.2	0.7	2.7 ± 0.1	8.1

(bottom), in which the actual thermal cycling time was only 27 min.

The extremely efficient heating properties of the MATCI (Table 2) make it remarkably suitable for rapid PCR. The exquisite temperature control should enhance specificity and sensitivity as it

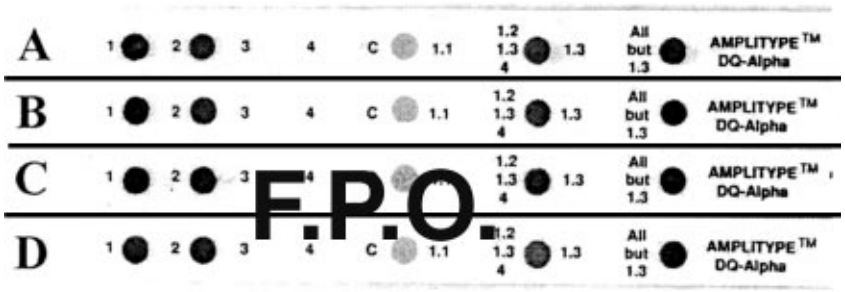


FIG. 3—*HLA DQα PCR using the MATCI. DNA was amplified with the Amplitype HLA DQα mix. Products were generated using thermal cycling parameters in Tables 1 and 2, and were characterized using reverse dot blot hybridization strips according to manufacturer's instructions.*

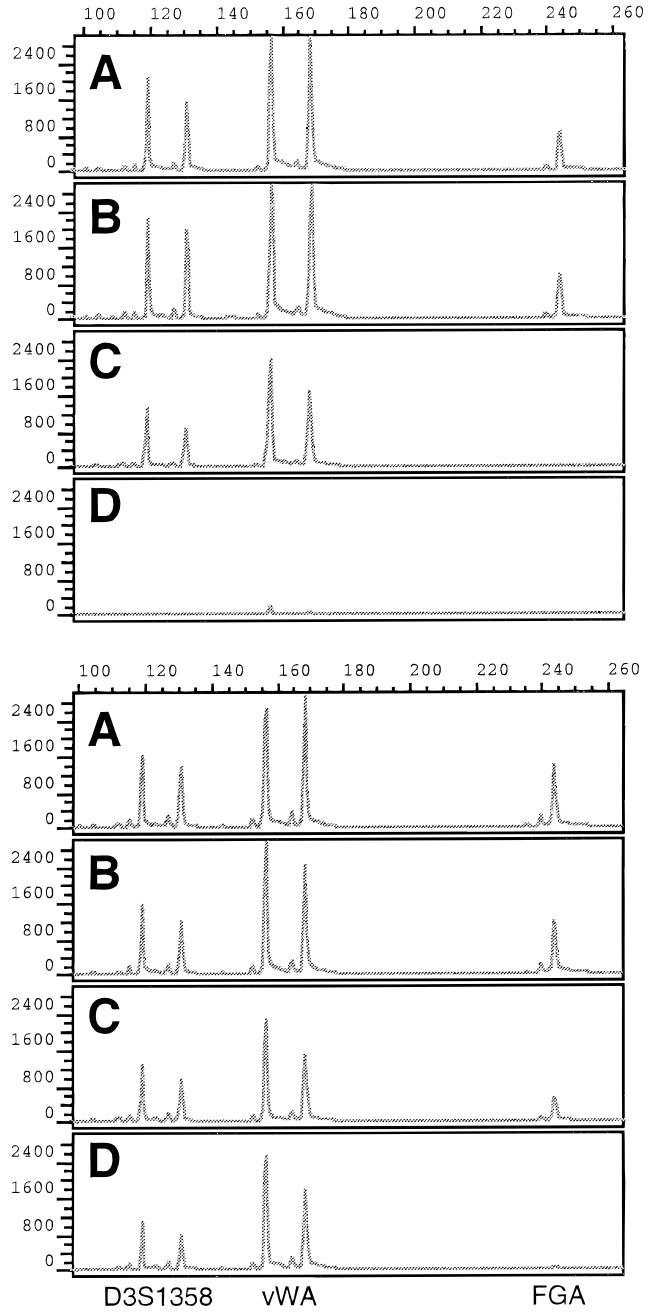


FIG. 4—*STR PCR using the MATCI. DNA was amplified with the AmpFISTR Blue STR system. Products were obtained using thermal cycling parameters A,B,C, and D in Tables 1 and 2, and were analyzed on an ABI 373 polyacrylamide slab gel electrophoresis system. STR loci are indicated. Top panels A-D, PCR Mix 1; Bottom panels A-D, PCR Mix 2.*

decreases nonspecific annealing of the primers. Nonetheless, distinguishing the alleles for each locus still requires benchtop equipment; therefore, further development to integrate PCR product characterization is desired. One possible scheme would involve performing DQα and PM typing using TaqMan fluorogenic hybridization probes (16,17) detected with the MATCI's solid-state optics system. Recently, TaqMan assays were performed on the MATCI to correctly distinguish different species of orthopoxvirus and two alleles of the human complement component C6 gene on the basis of a single nucleotide difference within the fluorogenic hybridization probe-binding sequence (Ibrahim MS et al., submitted to Anal. Chem., 1997). The potential to use the real-time detector for a fluorogenic probe-based DQα typing assay was demonstrated in Fig. 2; albeit, an actual assay would entail replacing the ethidium bromide with allele-specific fluorogenic probes. Another typing approach with the MATCI, as a consequence of previous work demonstrating the feasibility of integrating the PCR chip with a CE chip (13), would incorporate the separation and detection of STRs. An addressable oligonucleotide probe array is yet another conceivable analytical detection method (9–11).

Thus we have described the successful application of a prototype battery-powered instrument to perform rapid PCR for forensic identity testing purposes. The value of a multiple chamber MATCI in the crime lab would be enormous, and although there is the potential to use such an instrument at the crime scene, this application would be open to debate.

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