Idaho Incubation Fund Program

Final Report Form

Proposal No. **HERC IF12-003 As June 30th, 2012**
Name: **Dr. Greg Hampikian**
Name of Institution: **Boise State University**
Project Title: **Biological Testing with MSM Micropumps**

Information to be reported in your final report is as follows:

1. Provide a summary of overall project accomplishments to include goals/milestones met, any barriers encountered, and how the barriers were overcome:
   We constructed a pump suitable for incorporation in devices for mobile DNA profiling developed by the two leading companies (L and I) in the field. The cost of materials is still too high for full market penetration, but that is anticipated to drop as the materials themselves are not expensive. Present high costs are due only to the current preparation process.

2. Describe the current state of the technology and related product/service:
   See attached, submitted paper.

3. List the number of faculty and student participants as a result of funding:
   Faculty: 2 Graduate students: 1 Undergraduate students: 1

4. What are the potential economic benefits:
   The micropump market is growing as more medical, environmental, and forensic devices are being developed. We have established relationships with the market leaders for the forensic market. In order to continue serving and exploiting those relationships, we have formed a company, Response Magnetics, in Boise. The company will work with Boise State University, investors, and private sector corporations to license intellectual property being patented by Boise State University as a result of this project.

5. Description future plans for project continuation or expansion:
   Dr. Ullakko (now in Finland) who was part of our first HERC grant, is continuing pump development with Aaron Smith who was employed in both of our HERC grants. Aaron recently graduated from the BSU Engineering program, and is now working with Dr. Ullakko funded by grants from a public private partnership in Finland. Dr. Hampikian is taking a sabbatical and will use that time to further corporate ties between BSU and L and I, and will visit the lab in Finland.

6. Please provide a final expenditure report (attached) and include any comments here:

7. List invention disclosures, patent, copyright and PVP applications filed, technology licenses/options signed, start-up businesses created, and industry involvement:
   2 patents have been applied for which were developed from our HERC funding. We have an NDA with L, a fortune 500 US company, and have demonstrated the pump for them.
   We have a second NDA being completed with I, the first company with a commercial mobile forensic analyzer.

8. Any other pertinent information: See attached paper (below)

Magnetic shape memory micropump: Enhanced PCR, contact-free and compatible with human DNA profiling

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Magnetic Shape Memory (MSM) Ni-Mn-Ga elements are relatively new materials with a variety of remarkable properties. They respond to changes in magnetic fields by elongating and shortening up to 6%. We have constructed a micropump which consists principally of a single component, the MSM element. The pump can be driven by the rotation of a diametrically magnetized cylindrical magnet or by an electrical rotation of the magnetic field; it is reversible, and can be effectively operated by hand without any electrical power. The MSM element does not inhibit polymerase chain reaction but enhances it. We demonstrate that it is compatible with forensic applications and show that it improves human DNA profiling. This novel pump is suitable for lab-on-a-chip applications that require microfluidics.

Keywords: micro total analysis systems (μTAS), lab-on-a-chip, STR, Magnetic Shape Memory, Ferromagnetic Shape Memory, MSM, Twin Boundary, Magnetostriction, Ni_2MnGa, polymerase chain reaction, PCR, microfluidics, forensic DNA

Introduction

The lab-on-a-chip revolution has produced a number of innovations that herald a new age of portable field instruments and point of care diagnostics[1]. These micro total analysis systems (μTAS) have incorporated a variety of traditional material such as glass and silicone, but other materials are now being widely adopted such as ceramics and sapphire[2], polymers such as polydimethylsiloxane (PDMS)[3,4], polymethylmethacrylate (PMMA)[5,6], polycarbonate (PC)[7,8] and cycloolefins (COP)[9]. Many functional components for these miniaturized devices have been developed for biological testing, including homogenizers, cell disruptors, antigen binders, laser detectors, heaters, electrophoretic separators, mobility analyzers, etc.[10]; and there is a trend toward self-contained disposable chips for clinical use[11,12]. One area that has not seen as much development is that of micropumps[13], with many devices still being driven by external, traditional peristaltic pumps or syringes[12]. The field has seen the development of piezoelectric[14], lithographic[4], ferromagnetic fluid[15], acoustic wave[16] and polymeric pumps[17-20], but there is still large room for improvement in terms of a component-based chip technology, where pumps can be manufactured along with the chips and placed throughout the chip architecture. One desirable feature of any such micropump component is that it be free from electrical contacts and sealed within a potentially disposable lab-on-a-chip unit. Another feature of an ideal micropump is that, to the fullest extent possible, the pump material itself would pump (akin to muscle fibers) and

would be able to serve without ancillary mechanical devices. Finally, the pump must be both precise and sensitive over a wide range of volumes.

We have developed a pump matching these criteria, made of magnetic shape memory (MSM) elements consisting of Ni-Mn-Ga alloys[21,22]. We believe this is the first use of MSM alloys in biological testing, and we demonstrate that our pump, and the material itself, enhances polymerase chain reaction (PCR) and can be used to generate accurate forensic profiles using popular commercial kits (ABI, Quantifiler and Identifiler). Dimensions of MSM materials change when a magnetic field is affected on the MSM material[21,23]. The magnetic-field-induced strains can be over 10%[24]. The strains of MSM materials are two orders of magnitude larger than those of giant magnetostrictive materials, *e.g.*, Terfenol-D™. Magnetic-field-induced shape changes of magnetostrictive materials and MSM materials have been used for pumping fluids[25,26]. In the current study, we made an MSM pump that is based on the same pumping principle as Jokela's peristaltic design[25]. In this design, a shrinkage generated and moved by a local magnetic field transfers fluid.

Materials and Methods

Pump design

The pump was built to fit the specifications of standard forensic DNA profiling (generally 1-20 µl volumes), and to be capable of precisely delivering the solutions used in these reactions: DNA in water or complete profiling reactions with polymerase, nucleotides, genomic DNA, Magnesium, buffers and all the components of the manufacturer's kit. The pump is made of a Ni-Mn-Ga single crystal serving as an MSM element, suspended in an elastic resin and covered with a glass slide which is bonded to the element (with epoxy) at its ends. Two holes (1 mm in diameter) were previously drilled into the glass slide as inlet and outlet (see Fig. 1-3).

Ni-Mn-Ga crystal and element production

Magnetic shape memory $Ni_{51.3}Mn_{26.3}Ga_{22.4}$ (numbers indicate atomic percent, nominal composition) single crystals were grown via a modified Bridgman method using an oriented seed crystal aligned with $<100>_C$ parallel to the growth direction. The composition was chosen such as to provide the 10M martensite structure and a very low twinning stress. We have also made comparable pumps using MSM elements purchased from Adaptamat Ltd., Finland, but these were not used for this study.

Glass preparation

Two 1 mm diameter holes were cut into the glass microscope slides with a distance of 4 mm from the center of each hole. The glass slide was then cut using a diamond impregnated wafer saw so the dimensions were 25 mm x 10 mm x 1 mm. Ethanol was used to clean the surface of the glass to ensure there were no particles that could interfere with the bonding of the elastomer, the epoxy or the working surfaces of the Ni-Mn-Ga element.

MSM element preparation

The working mechanism of the MSM micropump is the Ni-Mn-Ga element. The MSM element was cut from a Ni-Mn-Ga single crystal using a wire saw (50 µm diameter wire) so that the overall dimensions were 20mm x 2.5mm x 1mm. To relieve surface stresses caused by cutting, the sample was then electropolished using a chilled solution consisting of 40 ml of ethanol and 20 ml of 16M nitric acid. After compressing the MSM element so that it was in a single martensite variant state[27], it was then electropolished four times for five second intervals at twelve volts. The MSM element was rinsed after each interval with a chilled sodium bicarbonate solution to cool the element and neutralize the acid.

The working surface of the MSM micropump requires special attention to make certain that the pump is sealed. Consequently, one surface of the MSM element requires mechanical polishing to ensure that it is planar. A 9 μm, 3 μm then 1 μm polishing slurry was used in conjunction with a polishing wheel and a polishing jig for five minutes at each interval. The MSM element and polishing jig were rinsed thoroughly with distilled water between each step to ensure there was no particulate contamination.

After the MSM element had been polished, it was carefully cleaned with ethanol and then compressed into a short, single phase and placed on a fixture with a rotating diametrically magnetized cylindrical N52 permanent magnet (K&J Magnetics, Inc) beneath it (Fig. 1).

Using the perpendicular magnetic field from the diametrically magnetized cylindrical magnet, a second phase was introduced into the middle of the MSM element that was approximately 1 mm in length. Without this alternate, long phase in the MSM element, the micropump would be unable to transfer liquid from inlet to outlet.

Micropump construction

A two part all-purpose epoxy (Devcon) was used to fix and constrain both ends of the MSM element onto the prepared glass slide (Fig. 1A and Fig. 2). It is important to align the center of the MSM element, both its length and width, to the two holes while leaving the intermediate, long variant undisturbed. While the epoxy was hardening, low pressure was applied to the back of the MSM element to make sure there was complete surface-to-surface contact between the glass and element.

After the epoxy was completely cured, a minimal amount of 100% silicone caulk was carefully applied to the corner created between the MSM element and the glass slide (Fig. 1B). Dow Corning Sylgard 184 silicone elastomer is a liquid before it cures, so the silicone caulk helps seal the working channel so that the elastomer won't leak into it and render the micropump ineffective. The silicone caulk was allowed to cure before preparing the elastomer.

A small piece of electrical tape was placed over the two exposed holes to make sure that the elastomer wouldn't leak into them. Dow Corning 92-023 primer was then painted onto only the glass that would be in contact with the elastomer. The primer acts as an adhesive between the elastomer and another surface and, for this reason, it must not get onto the MSM element. Doing so could suppress local straining of the MSM element.

The prepared micropump was then placed into the center of a polystyrene weigh dish. After thoroughly mixing the Sylgard 184 silicone elastomer as per its instructions, it was poured over the micropump so that the elastomer completely covered it, adding 0.5 mm to the total thickness of the micropump. The weigh dish was set aside and allowed 48 hours to cure at room temperature. Finally, the elastomer was cut to fit the glass plate. Overall, the micropump dimensions measured 25 mm x 10 mm x 2.5 mm.

DNA Profiling

The commercially available AmpF*l*STR® Identifiler® PCR Reagents kit was used to set up the reactions at a total volume of 25 µl (for the samples having contact with the pump) or 12.5 µl (for the positive and negative controls). Each 25 µl reaction contained 10.5 µl of the AmpF*l*STR® PCR Reaction Mix, 5.5 µl of the AmpF*l*STR® Identifiler® Primer Set, and 0.5 µl of the AmpF*l*STR® Kit AmpliTaq Gold® DNA Polymerase. Those reactions containing only 12.5 µl total volume contained all the listed reagents in exactly half that amount; these were first combined in a master mix, and then distributed in the appropriate amount to each sample tube. The control DNA from the AmpF*l*STR® Yfiler™ PCR Reagents kit (cell line 9947A) was used as the sample and positive control DNA, diluted to a final concentration of 0.1 ng/µl; 10 µl of which was added to the full (25 µl) reactions, 5 µl of which was added to the half (12.5 µl) reactions. The negative control had 5 µl of 1X TE (IDT; pH 8.0). All reactions

sat incubating at room temperature while the samples were put through the pump.

Four water washes were performed on the pump: first 10 µl, second 15 µl, and the third and fourth 20 µl of nuclease-free water (IDT). Water was added continuously through the pump (hand turning the cylindrical magnet). As the water came off the outlet well, it was wicked off using a Kimwipe™ (Kimberly-Clark). The pump was allowed to dry for approximately 5 minutes, until it was free of visible liquid. Then, the first sample (Pu 1) was added to the inlet well in the same manner as the water, until all 25 µl had been added to the inlet well. The sample was collected from the outlet well 1-2 µl at a time using a micropipette, until all liquid possible was extracted from the pump and placed in a fresh tube. The first 5 µl of Pu 1 were collected and stored separately from the rest of the output, to avoid any potential water carry-over from the earlier washes. The remaining two samples (Pu 2 and Pu 3) were put through the pump one at a time, without any washing of the pump between samples, and collected separately in the same way as Pu 1.

Once all three samples were run through the pump, 12.5 µl was collected from each pump sample tube and placed in a fresh tube for use on the thermal cycler. All samples were vortexed and spun down and placed on a Bio-Rad MJ Mini™ Personal Thermal Cycler using a protocol with the following conditions: 95°C for 11 min, 28 cycles of 94°C, 59°C, and 72°C for 1 minute each, and a final step of 60°C for 1 hour and 30 minutes. After PCR, the samples were loaded on the Applied Biosystems 3130 Genetic Analyzer and the results were examined using GeneMapper® ID-X software.

Quantifiler

The commercially available Applied Biosystems Quantifiler® Human DNA Quantification Kit was used with half reactions (12.5 µl volume total: 1 µl DNA (0.1 ng), 6.25 µl Quantifiler™ PCR Reaction Mix, 5.25 µl Quantifiler™ Human Primer Mix); the DNA used for these experiments was the positive control DNA from cell line 9947A (AmpF*l*STR® Yfiler Control DNA 9947A at 10ng/ µl) in a 1/100 dilution for a final concentration of 0.1 ng/ µl. There were three samples to be treated with the MSM element (RT1, 2, 3), and three positive controls (RPC1, 2, 3) to determine repeatability as well as a negative control (RNC). Samples were placed in an Applied Biosystems MicroAmp® Optical 96-Well Reaction Plate, where the MSM element was placed in the well of each RT sample for 10 minutes at a time. Elements that were re-used were cleaned with 10% bleach and 70% EtOH. Once all incubations were complete, the plate was sealed with an Applied Biosystems MicroAmp® Optical Adhesive Film and was centrifuged at 3000 rpm for 1 minute before loading onto the Eppendorf Mastercycler® ep Realplex 4. PCR conditions used were those recommended in the Quantifiler® Human Kit Manual: 50.0°C for 2 minutes, 95.0°C for 10 minutes, and then 40 cycles of 95.0°C for 15 sec followed by 60.0°C for 1 min. The Quantifiler® kit uses a dual hybridization Taqman® probe whose target gene is human telomerase reverse transcriptase (hTERT), the reporter dye being FAM. Standards were run in duplicate on the plate with the samples as recommended by Applied Biosystems in the kit manual using the concentrations listed therein. Results were analyzed using the realplex software, and a standard curve was produced, after eliminating one standard at each of the following concentrations: 0.068 ng/µl and 0.023 ng/µl, with a slope of -3.300, a Y-intercept of 28.91, and R^2 value of 0.996.

RESULTS

No loss of DNA after MSM pumping

We assessed the PCR amplification and final forensic profiles obtained from PCR reactions incubated for 10 minutes with the MSM element, the elastomer, and the silicone components of the pump assembly. While the silicone, and to a lesser degree the elastomer, had some negative impact on PCR (data not shown); Ni-Mn-Ga, the material of the only

essential pump part, enhanced amplification significantly. Encouraged by this, we used the assembled pump to assess its impact on PCR and profile quality. We prepared 6 samples of human genomic DNA in water (0.1 ng/µl) with Quantifiler reaction mix and primers; 3 of the samples were incubated for 10 minutes with Ni-Mn-Ga at room temperature, and three were left at room temperature as controls; a seventh reaction served as a reagent control, and had no genomic DNA. All samples were prepared in a 96-well plate, and amplified according to the manufacturer's guidelines. The samples that were incubated with the Ni-Mn-Ga amplified as well or better than the standard reaction controls (Fig. 5), indicating that the alloy does not decrease the measureable amount of DNA in aqueous solution, and that it does not produce a net negative effect on DNA amplification.

Improved forensic profiling following MSM pumping

We then prepared 6 PCR amplification reactions with genomic DNA (and one reagent control with no template DNA) in 0.2 ml plastic tubes, according to the Identifiler instructions. Three of the reactions with genomic DNA were run through our pump before placing them in new tubes. The remaining tubes were left at room temperature during this time. All 7 reactions were then placed in a thermocycler for amplification. We followed kit manufacturer's recommendation for all cycling parameters, using 0.5ng of the kit's genomic control DNA in a 12.5 µl total reaction volume.

The Identifiler results show that PCR reactions that were run through the pump were significantly enhanced (Figs. 6 and 7). For each allele, indicated by locus on the X axis (Fig. 7), the RFU areas are higher in the pumped (Pu) samples. In all experiments to date, we have found that PCR reactions that pass through the pump yield DNA profiles with higher DNA peaks than those which do not go through the pump. We are currently assessing the factors associated with the element that are required for this enhancement.

Conclusions and Discussion

We have demonstrated the design and use of a new micropump, essentially made from a single component, a Ni-Mn-Ga MSM element. This pump is compatible with biological testing, and improves PCR yield in forensic profiling. An added advantage of the pump is that it does not require any electrical or physical contact with the pump driver, and therefore can be a sealed and disposable component for clinical and field applications. We have operated the driving magnet by hand in all of the experiments shown here, but have also achieved more rapid pumping using an electric drill motor at 1000 RPM. Thus, the pump is suitable for field operations where power is unavailable (or undesirable) and where sealed disposable units are preferred. We are now using the elements to produce lab-on-a-chip designs with multiple, independent MSM pumps and are producing PCR enhancers based on this material.

There is a clear advantage in using materials that catalyze (or enhance) reactions for building μTAS components. Ni-Mn-Ga offers improved PCR and has other diverse properties which make it promising for an array of microdevice components. Its capacity to transduce charge, motion and current [28,22] make it ideal for actuators, energy harvesters and a variety of measuring devices. Finally, biological/metal hybrids have been constructed using other metal catalysts[29-32], but hybrids of Ni-Mn-Ga would combine the unique sensing and motility characteristics of Ni-Mn-Ga with the established biological properties of biomolecules, such as substrate specificity and biological catalysis.

FIGURES:

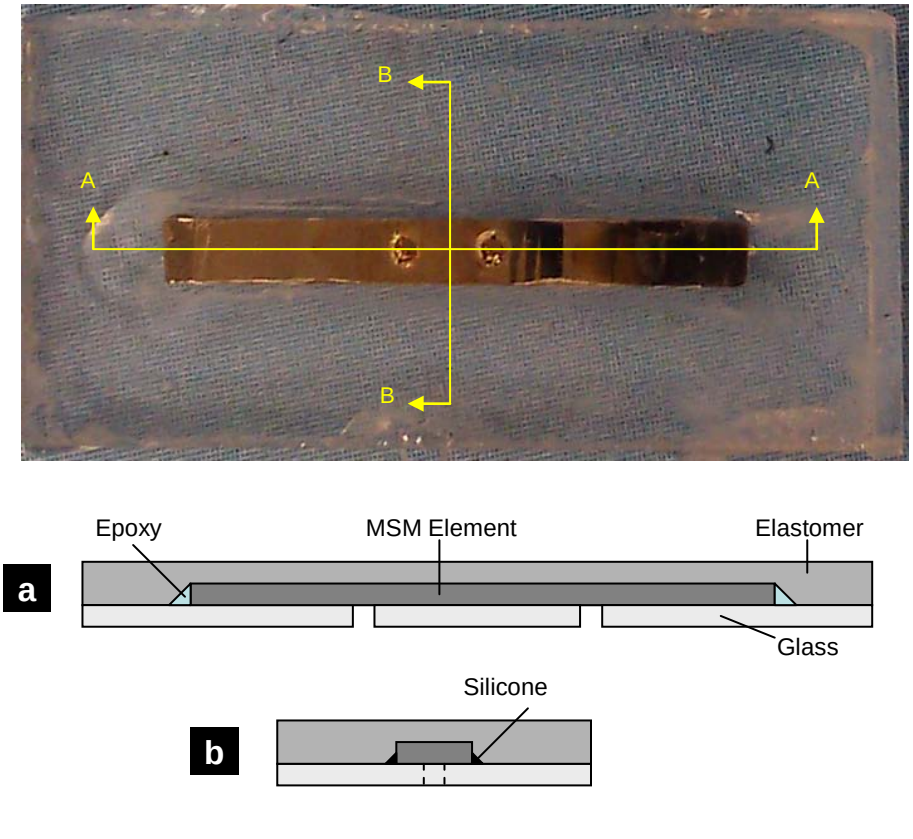

Fig. 1 Construction details of the MSM micropump. a, Epoxy is used to constrain both ends of the MSM element, and elastomer provides a sealing base (shown on top here, during construction). **b,** Minimal silicone is applied to the corner between the MSM element and the glass slide.

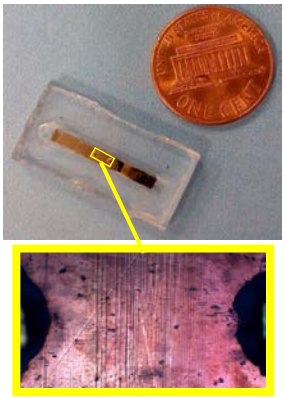

Fig. 2 MSM pump relative dimensions The working mechanism of the MSM micropump is within the yellow box. The inset is a close-up of the MSM element as it pumps a red aqueous solution.

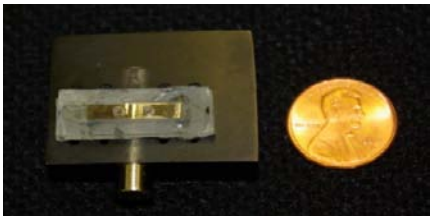

Fig. 3 MSM pump in jig with diametrically magnetized cylindrical magnet.

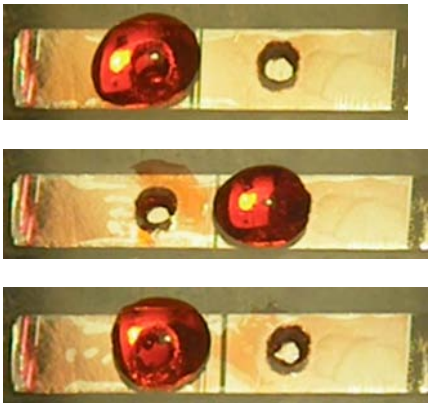

Fig. 4 MSM micropump moving colored aqueous solution
Magnified view:
Initial condition *(Top)*
Fluid pumped right *(Mid)*
Fluid pumped back *(Bottom)*
(See appendix for video)

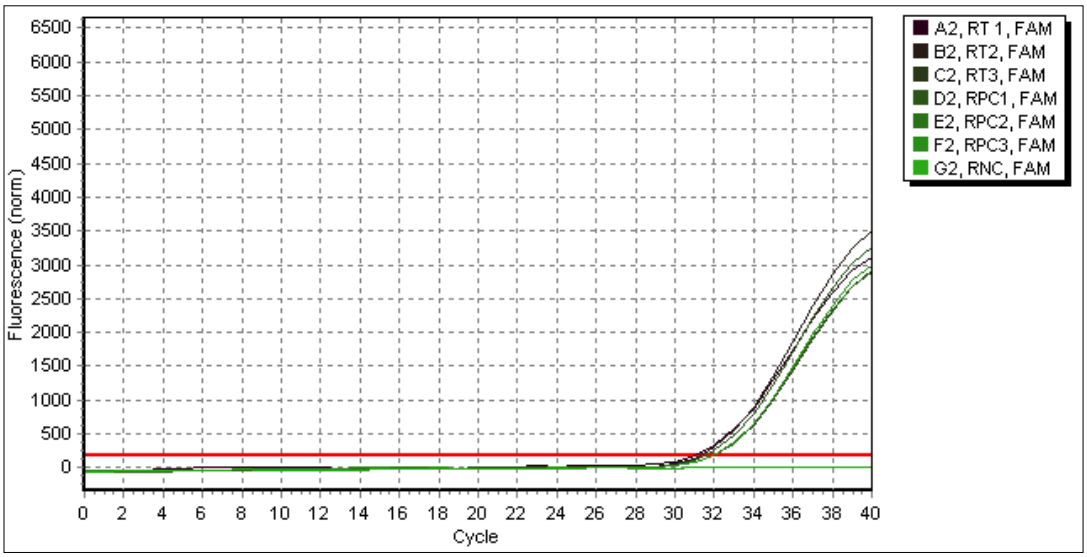

Fig. 5 Quantity of amplifiable DNA in MSM incubated samples is equal to higher than that in MSM-free controls. Visualization of the polymerase chain reaction in samples treated with MSM versus untreated (controls). RT samples are those incubated with MSM element for 10 minutes; RPC samples were reaction controls used for comparison. RNC is the reagent control (no DNA template). All samples had quantification cycles (Cq) between 31.13 and 32.04, except for the negative control (which never crossed the threshold): RT1 31.13; RT2 31.43; RT3 31.97; RPC1 31.71; RPC2 32.04; and RPC3 32.00. All samples incubated with the MSM element amplified as well or better than the controls. The baseline (green line at 0 fluorescence) represents the negative control (no template DNA). The red line is the threshold (10 times the standard deviation above the noise of the baseline) determined by the Realplex software for calculating the quantification cycles (Cq).

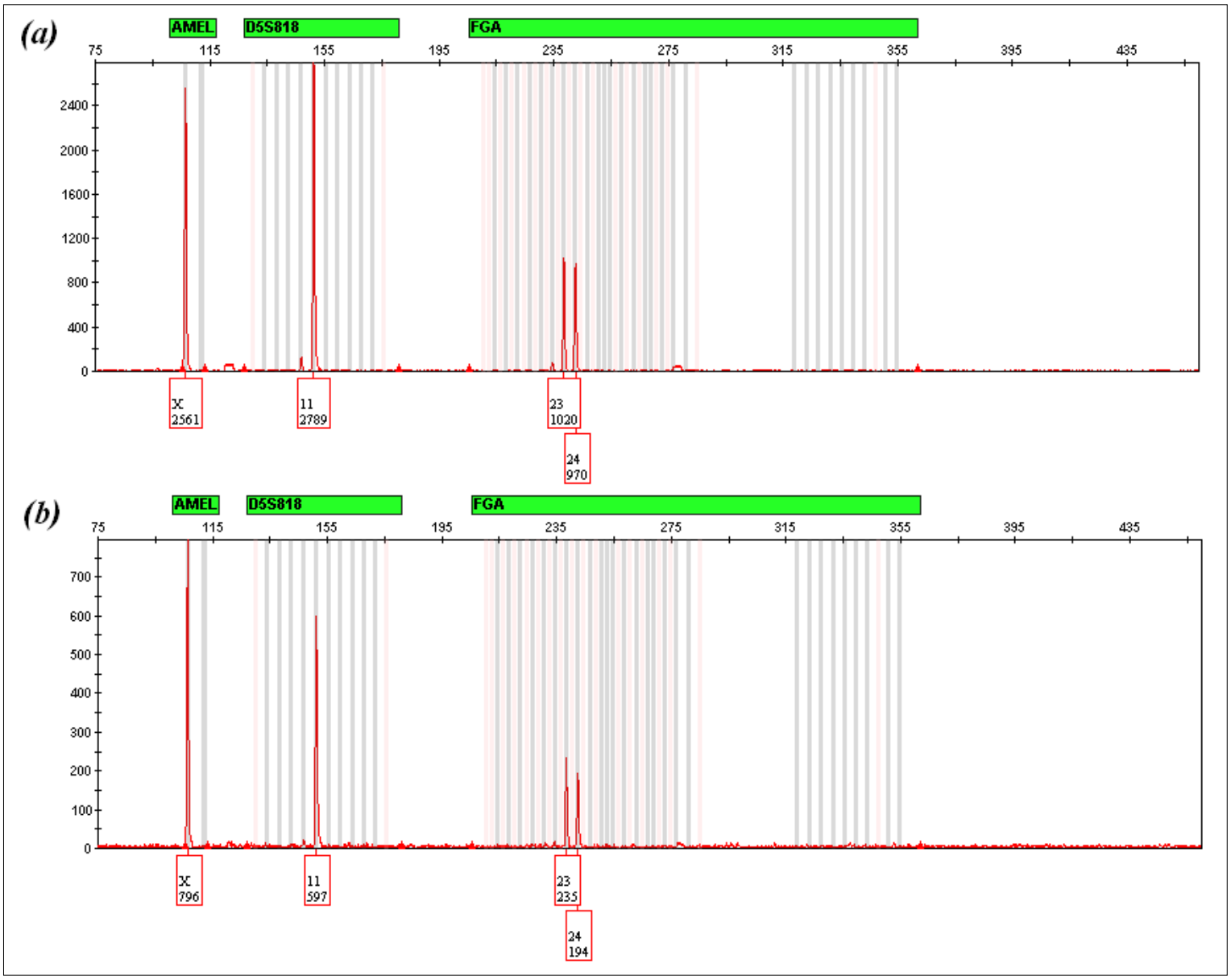

Fig. 6 Forensic DNA Profiling enhanced by MSM pumping. PET panel of MSM pumped sample (a) vs Control (b). Alleles at loci Amelogenin, D5S818, and FGA for samples pumped through the MSM micropump (a) and the standard reaction (b). The x axis is size in base pairs of STR fragments, and the y axis is peak height in relative fluorescence units (RFU). Each allele is labeled by allele number and peak height in RFUs, respectively. These panels are representative of typical results observed in the pump experiments (see Fig. 7).

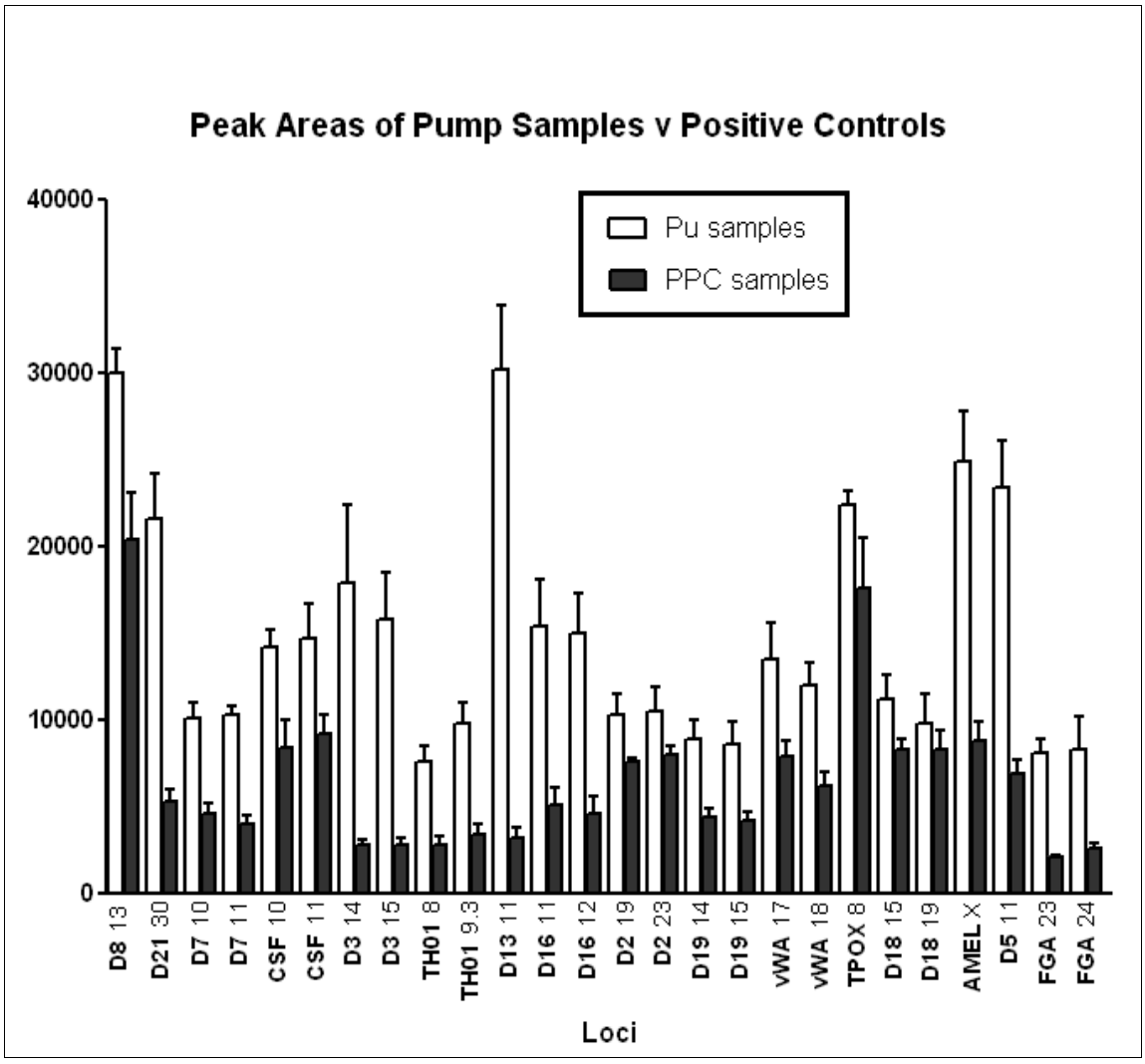

Fig. 7 MSM Pump improves PCR yield in forensic kit profiles. This graph shows the 16 genetic loci assessed by the forensic profiling kit, Identifiler. The bars are labeled by locus name (bold) and the allele size. A single master PCR reaction was split into 7 tubes. Three samples (Pu1, 2, 3) were run through the MSM element pump; while three other samples (RPC1, 2, 3), and the reagent controls were not. PCR was performed, and the samples were analyzed by capillary electrophoresis. Sample peak areas were recorded, producing the results depicted here. Profiles were correctly called in all reactions, and the peaks areas were enhanced by MSM pumping.

Acknowledgements
This work was funded by the Idaho State Board of Education Higher Education Research Council (HERC), the Defense Threat Reduction Agency under contract number W81XWH-07-1-000, DNA Safeguard, and the Department of Energy, Office of Basic Energy Science (K.U. and P.M.) through contract No. DEFG-02-07ER46396.

References

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Appendices:

1. Video of pump in action

FINAL EXPENDITURE REPORT

A. FACULTY AND STAFF		
Name/Title	$ Amount Requested	Actual $ Spent
B. VISITING PROFESSORS		
Name/Title	$ Amount Requested	Actual $ Spent
C. POST DOCTORAL ASSOCIATES/OTHER PROFESSIONALS		
Name/Title	$ Amount Requested	Actual $ Spent
Mike Davis – Research Assistant	$26,500.00	$10,149.00
D. GRADUATE/UNDERGRADUATE STUDENTS		
Name/Title	$ Amount Requested	Actual $ Spent
Carolyn Failla – Undergraduate Student	$4,000.00	$370.00
Aaron Smith – Graduate Student	$4,000.00	$2,592.60
E. FRINGE BENEFITS		
Rate of Fringe (%)	$ Amount Requested	Actual $ Spent
9% - Undergradaute Student, 1% Graduate Student, 56% Research Assistant	$8,103.00	$5,243.63
PERSONNEL SUBTOTAL:		$18,355.23
F. EQUIPMENT: (List each item with a cost in excess of $1000)		
Item/Description	$ Amount Requested	Actual $ Spent
1. Crossington Manual Sputterer + High Speed Rotary Pump for Sputterer	$0.00	$7,775.43
2.		
3.		
4.		
EQUIPMENT SUBTOTAL:		$7,775.43
G. TRAVEL		
Description	$ Amount Requested	Actual $ Spent
1.	$2,000.00	$0.00
2.		
3		
TRAVEL SUBTOTAL:		

H. PARTICIPANT SUPPORT COSTS:		
Description	$ Amount Requested	Actual $ Spent
1.		
2.		
3		
PARTICIPANT SUPPORT COSTS SUBTOTAL:		

I. OTHER DIRECT COSTS:			
Description		$ Amount Requested	Actual $ Spent
1. General Lab Supplies		$5,397.00	$23,835.59
2.			
3.			
OTHER DIRECT COSTS SUBTOTAL:			$23,835.59
TOTAL COSTS (Add Subtotals):			$49,966.25
TOTAL AMOUNT REQUESTED:			$50,000.00
TOTAL AMOUNT SPENT:			$49,966.25