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A MEMS-Based Approach to Single Nucleotide Polymorphism Genotyping

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Abstract

Genotyping of single nucleotide polymorphisms (SNPs) allows diagnosis of human genetic disorders associated with single base mutations. Conventional SNP genotyping methods are capable of providing either accurate or high-throughput detection, but are still labor-, time-, and resource-intensive. Microfluidics has been applied to SNP detection to provide fast, low-cost, and automated alternatives, although these applications are still limited by either accuracy or throughput issues. To address this challenge, we present a MEMS-based SNP genotyping approach that uses solid-phase-based reactions in a single microchamber on a temperature control chip. Polymerase chain reaction (PCR), allele specific single base extension (SBE), and desalting on microbeads are performed in the microchamber, which is coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to analyze the SBE product. Experimental results from genotyping of the SNP on exon 1 of the HBB gene, which causes sickle cell anemia, demonstrate the potential of the device for rapid, accurate, multiplexed and high-throughput detection of SNPs.

Keywords

Genotyping; Microfluidics; Single Nucleotide Polymorphisms; Bead-Based Single Base Extension; Bead-Based Polymerase Chain Reaction

1. INTRODUCTION

Genetic mutations take many forms, ranging from chromosome anomalies to single-base substitutions [1]. Among them, single nucleotide polymorphisms (SNPs), which are single nucleotide variations in the genome between different individuals, are the most common form, occurring approximately once every 1000 bases [2]. SNPs can be used as genetic markers to identify genes associated with complex disease [3, 4]. Therefore, accurate identification of SNPs is of great utility to disease diagnosis and prognosis [5-7].

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Genotyping of SNPs can be based on enzymatic cleavage, allele specific hybridization, allele specific ligation or cleavage, and allele specific primer extension [8]. Enzymatic cleavage, illustrated in Invader® Assays [9, 10], utilize thermostable flap endonucleases (FEN) and fluorescence resonance energy transfer (FRET) to recognize and detect SNP by the annealing of allele-specific overlapping oligonucleotides to the target DNA. While it is highly accurate, this method is generally time-consuming and difficult to multiplex (i.e., to detect multiple SNPs in one reaction). Allele-specific hybridization, used for example in the Affymetrix® Human SNP Array [11], is based on matched or mismatched probes annealing to the target DNA sequence adjacent to the SNP site [7]. This method allows detection of up to 2 million SNPs simultaneously, but it requires labor-intensive genomic DNA (gDNA) digestion, fractionation and amplification, and is prone to unspecific binding that leads to false positives [12]. Allele-specific ligation or cleavage combines the hybridization of probes specific to the target DNA sequence and the specificity of the ligase enzyme (e.g., SNPLEX™ Assay [13]) or 5' nuclease (TaqMan® Assay [13]) to distinguish matched or mismatched nucleotides. These assays have a very high level of specificity, but their applicability is hindered by slow reaction speeds and the limited number of modified probes available [8]. Allele-specific primer extension, which involves allele-specific incorporation of nucleotides in primer extension [8, 14], is an emerging SNP genotyping method that offers flexibility and robustness. The discrimination between different incorporated nucleotides is based on either fluorescence (e.g., Illumina® Infinium Assays [15]) or mass spectrometry (e.g., Sequenom® iPLEX Assays [16]). While rapid, accurate and capable of multiplexing, they require complicated design of primer and fluorescent-tagged or mass-tagged nucleotide [17, 18]. There is hence a strong need for new genotyping platforms to address these issues.

Microfluidics technology can potentially enable fast, low-cost, and automated SNP detection with improved sensitivity, resolution, accuracy, efficiency and throughput, as well as minimized sample consumption [19]. The afore-mentioned genotyping principles have been implemented in microfluidic systems to generate modified DNA indicative of SNPs, which is then detected by methods such as electrophoresis and bioluminescence [19, 20]. Electrophoresis, in which an applied electric field separates DNA fragments by charge-to-mass ratio, provides high accuracy with reduced buffer and sample consumption, but has limited potential in high throughput and multiplexed genotyping [21]. Alternatively, bioluminescent methods utilize fluorescent probes to provide either high sample throughput [22-25] or high accuracy [26] along with enhanced sensitivities and reduced time, sample and reactant consumption, but are limited by false positives and an inability to multiplex. Therefore, more research is needed to develop microfluidic SNP detection systems that offer improved accuracy, ability to multiplex, and increased throughput.

This paper presents a MEMS-based SNP genotyping approach using solid-phase-based reactions in a single microchamber on a temperature control chip. Polymerase chain reaction (PCR), allele specific single base extension (SBE) and desalting on microbeads are performed in the single microchamber, and are coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to identify SBE products [27]. The use of SBE and MALDI-TOF MS provides the possibility of accurate, fast and multiplexed detection [28-30]. The single chamber design eliminates the use of multiple microchambers and valves, resulting in greatly simplified fluid handling, and integration of PCR with SBE on-chip has the potential to improve assay efficiency. Miniaturization reduces sample and reagent consumption, and when implemented in array format, this integrated SNP detection approach can potentially allow parallelized and high-throughput analysis of patient samples. We demonstrate this MEMS-based SNP genotyping approach by detection of the SNP on exon 1 of the HBB gene located in chromosome 11,

which alters the sixth amino acid in the beta hemoglobin to cause sickle cell anemia, a life-threatening disorder [31].

2. PRINCIPLE AND DESIGN

2.1. Principle of MEMS-Based SNP Genotyping

Our MEMS-based SNP genotyping method performs PCR, SBE, and desalting reactions on microbeads in a single microchamber. The chamber is first packed with polymer microbeads that are functionalized with reverse primers via a biotin-streptavidin link and mixed with the target template DNA and PCR reagents, followed by thermal cycling to generate double-stranded DNA (dsDNA) (Figure 1A and Figure 1B), which is then purified by a buffer wash (Figure 1C). By introducing NaOH solution, template single-stranded DNA (ssDNA) is chemically eluted, leaving ssDNA complementary to the template on the microbeads (Figure 1D) to be analyzed in the following steps. Subsequently, SBE reactants are introduced, and allele-specific primers anneal to the complementary ssDNA immediately adjacent to the polymorphic site. These primers then undergo SBE, by thermally cycling the reaction mixture in the presence of dideoxynucleotides (ddNTPs) and enzyme, to generate primers extended by only one base (Figure 1E) [30]. Washing with DI water then removes free primers, salts and any other impurities for purification of the bead-bound extended and unextended allele-specific primers (Figure 1F). The additional nucleotide is then identified according to the difference in mass between the extended and unextended primers.

2.2. Design and Fabrication

The microfluidic device used to demonstrate the bead-based SNP genotyping approach consists of a polydimethylsiloxane (PDMS) microchamber situated on a temperature control chip integrated with a micro heater and temperature sensor (Figure 2A and B). The tapered microchamber (Figure 2A and B, 150 μm in height) with an approximately 5 μL volume contains dam-like structures (Figure 2A and B, 15 μm in height), called weirs, to retain microbeads (50 - 80 μm in diameter) during wash steps. The surfaces of microchamber are coated with Parylene C to prevent evaporative loss of reactants [32]. On the temperature control chip, a resistive sensor (16.5 mm L $\times$ 50 μm W) is located beneath the center of the chamber, and a resistive serpentine-shaped heater (296 mm L $\times$ 500 μm W) surrounds the temperature sensor. Thus, the chamber is heated with its temperature near the center measured by the sensor to complete a closed-loop temperature control setup.

The temperature control chip was first fabricated using standard microfabrication techniques. Briefly, a glass slide (Fisher HealthCare, Houston, TX) was cleaned by piranha. Chrome (10 nm) and gold (100 nm) thin films were deposited by thermal evaporation and patterned by wet etching. Then, a passivation layer of 1 μm of silicon dioxide was deposited using plasma-enhanced chemical vapor deposition (PECVD). Finally, the on-chip temperature sensor and heater were equipped with contact pads (2.5 mm $\times$ 2.5 mm, also fabricated from gold), which were opened by etching the oxide layer using hydrofluoric acid and connected to measurement instruments via wire bonding (Figure 2C).

In parallel, the microfluidic chamber was fabricated from PDMS (Sylgard 184, Dow Corning Inc. Midland, MI) using soft lithography techniques. SU-8 photoresist (MicroChem Corp., Newton, MA) was spin-coated and patterned on a silicon wafer to form mold-defining microfluidic features. Next, a PDMS prepolymer solution (base and curing agent mixed in a 10:1 ratio) was cast onto the mold and cured on a hotplate at 72 $^{\circ}\text{C}$ for 1 hour (Figure 2D).

Subsequently, the inlet and outlet were punched on the resulting sheet bearing the microfluidic features, which was then bonded to the temperature control chip after treatment of the bonding interfaces with oxygen plasma for 15 seconds (Figure 2E).

Finally, the surface of the microchamber was coated with a thin layer of Parylene C via chemical vapor deposition (Figure 2F), prior to packing streptavidin beads (Figure 2G). An image of a fabricated device is shown in Figure 3.

3. EXPERIMENTAL

3.1. Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. Streptavidin beads (Pierce Streptavidin Plus UltraLink Resin) were obtained from Thermo Fisher Scientific Inc. (Rockford, IL). Dideoxynucleotide triphosphates (ddNTPs) were purchased from Jena Bioscience GmbH (Jena, Germany). Deoxynucleotide triphosphates (dNTPs) and GoTaq Flexi DNA Polymerase were obtained from Promega Corp. (Madison, WI). Thermo Sequenase was purchased from GE Healthcare (Piscataway, NJ). Template DNA, including a mutated type (5'-CCT CAC CAC CAA CTT CAT CCA CGT TCA CCT TGC CCC ACA GGG CAG TAA **CGG CAG ACT TCT CCA** CAG GAG TCA GAT GCA CCA TGG TGT CTG TTT GAG GTT GCT AGT GAA CAC AGT TGT GTC AGA AGC AAA TGT AAG CAA TAG ATG GCT CTG CCC TGA CT-3', the SNP site is underlined and SBE primer annealing site is italic) and an unmutated type (5'-CCT CAC CAC CAA CTT CAT CCA CGT TCA CCT TGC CCC ACA GGG CAG TAA **CGG CAG ACT TCT CCT** CAG GAG TCA GAT GCA CCA TGG TGT CTG TTT GAG GTT GCT AGT GAA CAC AGT TGT GTC AGA AGC AAA TGT AAG CAA TAG ATG GCT CTG CCC TGA CT-3', the SNP site is underlined and SBE primer annealing site is italic) of the HBB gene, double biotin modified reverse primer (5'-double biotin-AGT CAG GGC AGA GCC ATC TA-3'), fluorescein (FAM) modified forward primer (5'-FAMCCT CAC CAC CAA CTT CAT CC-3', M.W. = 6651), and SBE primer (5'-ACG GCA GAC TTC TCC-3', M.W = 4513) were synthesized and purified by Integrated DNA Technologies (Coralville, IA).

3.2. Experimental Setup

Closed-loop temperature control of the microchamber was achieved using the integrated temperature sensor, heater, and the fan under the temperature control chip with a proportional-integral-derivative (PID) algorithm implemented in a LabVIEW (National Instruments Corp., TX) program on a personal computer. The resistance of the sensor was measured by a digital multimeter (34420A, Agilent Technologies Inc., CA), and the heater and fan were connected to two DC power supplies (E3631, Agilent Technologies Inc., CA) respectively. The inlet was connected to a syringe that contained reaction buffer or washing buffer driven by a syringe pump (KD210P, KD Scientific Inc., MA). The outlet was connected to a microcentrifuge tube for collection of genotyping product to MALDI-TOF MS or experimental waste. All fluorescent images of beads were taken using an inverted epifluorescence microscope (Diaphot 300, Nikon Instruments Inc., NY) with a CCD camera (Model 190CU, Micrometrics, NH), after removing the device from the fan (Figure 4).

3.3. Experimental Procedure

Just prior to experimentation, the streptavidin beads in the microchamber were rinsed with binding and washing (B&W) buffer (5 mM Tris-HCl, 0.5 mM EDTA, 1 M NaCl, and 0.01% Tween 20, pH=7.5). The reverse primer (50 pmol) in B&W buffer was introduced and incubated with the beads for 30 min, followed by washing with B&W buffer at 10 μ L/min for 10 min.

Bead-based PCR was performed for 30 thermal cycles as follows: 95 °C for 15 s, 56 °C for 30 s, and 72 °C for 30 s. A 5 µL sample of PCR reactants was introduced twice, prior to cycling and between 15th and 16th cycle, and each sample consisted of 0.08 pmol of template, 8.33 pmol of forward primer, 1× GoTaq Flexi Buffer, 0.83 units of GoTaq Flexi DNA Polymerase, 1.67 nmol of dNTP and 6.25 nmol of MgCl₂ (1.25 mM). The microbeads were then rinsed with 0.15 mM NaOH in B&W buffer at 5 µL/min for 10 min to elute template ssDNA, followed by a rinse of pure B&W buffer at 10 µL/min for 10 min, leaving complementary ssDNA on the beads.

To perform SBE, the SBE primer targeting the SNP on the complementary sequence of exon 1 of the HBB gene was extended by a single base in the microchamber using ddNTPs. A 5 µL sample of SBE reactants was introduced to the microchamber twice, prior to SBE and between 5th and 6th thermal cycle, and underwent 10 thermal cycles as follows: 90 °C for 15 s, 40 °C for 30 s, and 70 °C for 30 s. Each SBE reactant consisted of 6.67 pmol of primer, 16.67 pmol of ddNTP, 1× Thermo Sequenase reaction buffer and 2.67 units of Thermo Sequenase.

The microchamber was then rinsed using B&W buffer at 5 µL/min for 10 min, followed by desalting with DI water at 5 µL/min for 20 min. Finally, the microchamber was incubated at 95 °C for 1 min, followed by a rinse with DI water at 20 µL/min and 95 °C for 3 min, to elute the hybridized primer.

4. RESULTS AND DISCUSSION

This section presents and analyzes experimental results from the fabricated MEMS-based SNP genotyping device. We first characterize the temperature control chip. Then, bead-based PCR, chemical elution, *in-situ* desalting and thermal elution are performed using exon1 of the HBB gene to characterize each functional unit. Finally, the integrated procedure is executed on-chip to demonstrate successful SNP genotyping.

4.1. Temperature Control Characterization

The temperature-resistance relationship of the thin-film gold temperature sensor was calibrated following fabrication. The experimental data showed that the measured resistance (R) of the sensor exhibited a highly linear relationship with temperature (T), which can be fitted to $R=R_0[1+\alpha(T-T_0)]$, where R_0 is the sensor resistance at reference temperature T_0 , and α is the temperature coefficient of resistance (TCR) of the sensor. The TCR was determined to be $3.06 \times 10^{-3} \text{ } ^\circ\text{C}^{-1}$ for a typical chip, which had a reference resistance of 83.44 Ω at a reference temperature of 21.9 °C. Time-resolved tracking of on-chip thermal cycling showed that the chamber temperatures attained specified setpoints via control of the on-chip heater and off-chip fan quickly and precisely (Figure 5). The thermal time constant of a typical temperature control chip was 126 s based on an exponential fit. The time constants of closed loop temperature control (based on an exponential fit) were 1.4s for heating from 56°C to 72°C, 1.9 s for heating from 72 °C to 95 °C and 8.7 s for cooling from 95 °C to 56 °C, which represented a significant improvement over typical time responses of conventional PCR thermal cyclers (e.g., 6s for heating from 56°C to 72°C, 8 s for heating from 72 °C to 95 °C, and 16 s for cooling from 95 °C to 56 °C for the Eppendorf Mastercycler® Personal used in our related experiments below).

4.2. Characterization of Bead-Based PCR and Chemical Elution

To characterize bead-based PCR, reactants were thermally cycled on-chip and fluorescent bead intensity was then measured and compared to control tests. To obtain consistent results under controlled experimental conditions, template DNA was used as the target sequence for

the characterization. After B&W buffer washing, the fluorescent intensity of beads was significantly higher than those without thermal cycling, enzyme or templates, which were only 5%, 7% and 16% of the original test (Figure 6A). This indicates that the bead-based PCR process did amplify template DNA and that the fluorescently modified primer enables monitoring of this step of the SNP genotyping procedure [33].

Prior to SBE, template ssDNA generated during PCR must be removed from the bead-bound complementary DNA. To test the efficiency of the chemical elution method, the template ssDNA was first amplified using fluorescently labeled forward primers and double biotinylated reverse primers in a conventional thermal cycler, and the amplification product was immobilized onto the streptavidin beads, which were packed in the microchamber afterwards. The fluorescent intensity of the beads was then measured before and after rinsing with buffer containing 0.15 mM NaOH. The fluorescent intensity of rinsed beads was 87% lower than that of pre-elution beads (Figure 6B), indicating that most template ssDNA had been removed from the bead surface. To further demonstrate that the template ssDNA had been removed from the beads, rather than the dsDNA, 5 μ L of 5 μ M FAM-modified forward primers in 1 $\times$ PCR buffer was introduced into the microchamber. After incubating at 56 $^{\circ}$ C for 1 min, followed by washing with B&W buffer, the fluorescent intensity of the beads was similar to that before introduction of the NaOH elution (Figure 6B), which suggested that complementary ssDNA remained bound to the beads following the elution of template ssDNA. These results indicate a sufficiently high on-chip chemical elution efficiency using NaOH.

4.3. Verification of Thermal Elution and *In-Situ* Desalting

To generate a DNA solution prior to detection with MALDI-TOF MS, hybridized primers must be desalted and then thermally eluted into DI water. The effect of desalting and the efficiency of the thermal elution method were tested to ensure that DNA loss during this step would not compromise detection by MS. The fluorescently labeled forward primer in B&W buffer was first hybridized to the ssDNA on the beads and desalted with DI water. The fluorescent intensity of the beads was then measured before and after rinsing at 95 $^{\circ}$ C, and the elution product was manually pipetted to a MALDI plate and tested using MALDI-TOF MS. During desalting, the microchamber was rinsed with DI water, and the fluorescent intensity remained at 95.5% of pre-desalting intensity (Figure 7A). The chamber temperature was then elevated to elute hybridized primers. After elution, the fluorescent intensity of the beads was only 26% of pre-desalting intensity and 28% of pre-elution intensity (Figure 7A). To control for effects of temperature on fluorescent intensity, fluorescently labeled microbeads were heated in the thermal cycler for different durations. As shown in Figure 7B, it was obvious that heating for 4 min did not generate a noticeable change in fluorescent intensity, which showed that the intensity of the fluorescent label was stable in response to elevated temperatures, and that elution of primers was indeed the reason for the decrease in fluorescent intensity. Furthermore, following MALDI-TOF MS, a distinct mass spectral peak at 6651 m/z (Figure 7C) indicated effective desalting efficiency, showing the promise of using this method to desalt and elute genotyping products. The repeated experiments have shown similar results, from all of which the mass spectral peaks can be recognized consistently. These results demonstrate effective *in-situ* desalting and efficient thermal primer elution.

4.4. Integrated SNP Detection

Having tested the individual procedures necessary for SNP detection, the procedures were integrated and the SBE products were analyzed using MALDI-TOF MS. Theoretically, the mass of extended primer can be calculated according to the equation $m_p = m_t + m_n - m_b$, where m_p is the mass of extended primer, m_t is the mass of unextended primer, m_n is the

mass of corresponding ddNTP and m_b is the mass of bond formation (175 m/z). We detected SNPs on both mutated HBB gene and unmutated HBB gene. As the target nucleotides of the mutated and unmutated template DNA are adenosine and thymidine, a single dideoxyadenosine triphosphate (ddATP, M.W. = 472) and dideoxythymidine triphosphate (ddTTP, M.W. = 463) were incorporated into each primer, respectively. Thus the mass of the product for mutated and unmutated HBB gene were respectively expected to be 4810 Daltons (4513+472-175), as shown by the distinct peak at 4810 m/z in Figure 8A, and 4801 Daltons (4513+463-175), as shown by the peak at 4801 m/z in Figure 8B. The peak located at 4513 m/z in both Figure 8A and 8B was induced by unextended primers, which would not compromise the identification of the nucleotide at a SNP site. Repeated experiments on genotyping of both mutated and unmutated HBB gene has shown similar mass spectra consistently, indicating successful SNP detection.

5. CONCLUSION

We have developed a MEMS-based genotyping approach by incorporating PCR, SBE and desalting using solid-phase-based reactions in a single microfluidic chamber, and coupling this method to MALDI-TOF MS. The device consists of a microchamber that is situated on a temperature control chip integrated with a temperature sensor and heater, and is equipped with weirs to retain microbeads during wash steps. The microchamber surfaces are coated with Parylene C to prevent sample loss. The integrated temperature sensor and heater allow closed-loop temperature control for thermal cycling and thermally induced primer elution. By employing such solid-phase-based reactions, our approach can significantly simplify fluid handling by avoid utilizing any on-chip valves, and hence potentially be applicable for high-throughput assays.

Our experiments have shown efficient on-chip thermal cycling using the device design, which enables effective bead-based PCR, SBE and thermal elution. In addition, successful chemical elution using NaOH has been achieved. Subsequently, the capability of the device to perform *in-situ* desalting using solid-phase-based reactions was demonstrated. Finally, genotyping of SNPs on both a mutated and an unmutated HBB gene using the presented device was performed, coupled with MALDI-TOF MS. The nucleotides at SNP sites have been successfully recognized, although a 100% nucleotide incorporation was not yet achieved and could be addressed by running a larger number of cycles and introducing more effective mixing during thermal cycling.

In addition to successfully detecting an individual SNP, these results also indicate the compatibility of our approach with multiplexed genotyping. For example, if multiple primers are used to perform an extension simultaneously, each primer can detect a different SNP. Because the maximum molecular weight of ddNTPs is 488 Daltons (dideoxyguanosine triphosphate, ddGTP), by ensuring that the primers are designed with a mass difference of at least 500 Daltons, the mass spectrum of each different primer and extended products will not overlap. As a result, different mass spectral peaks can be recognized in the same spectrum to detect multiple SNPs. Such multiplexed SNP genotyping will be studied in future work.

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Biographies

Jing Zhu received his B.E. degree in Electronic Engineering and M.S. degree in Biology from Tsinghua University, Beijing, China, in 2005 and 2008 respectively. From 2008 to

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Mirko Palla received his B.S. degree in Mechanical Engineering from Clarkson University at Potsdam, NY in 2007. From 2007 to 2008 he conducted research as an R&D Engineer in George Church's laboratory at Harvard Medical School to develop a next generation DNA sequencing instrument (Polonator). He obtained his M.S. in Mechanical Engineering from Columbia University at New York, NY in 2010. He is currently pursuing a Ph.D. in Mechanical Engineering at Columbia focusing on novel DNA sequencing technology development. His research interests include microfluidics, plasmonic nanostructures and surface enhanced Raman spectroscopy with a focus on sequencing and single-molecular detection systems.

Stefano Ronca was born in Manerbio, Italy in 1986. He received his B.S. in Automation Engineering from Brescia University, Italy in 2008. Subsequently, he completed his M.S. in Mechanical Engineering at Columbia University in 2009, focusing on energy systems with a particular interest on renewable energies. In 2012 he received his second M.S. in Automation Engineering from Brescia University, completing his graduation thesis in the BioMEMS laboratory of Columbia University.

Ronald Wapner, who received his M.D. in Jefferson Medical College, is the professor in the Department of Obstetrics and Gynecology at Columbia University, and the director of Division of Maternal Fetal Medicine, New York-Presbyterian Hospital. Dr. Wapner is a nationally known clinician specializing in first trimester screening and invasive procedures. He has also authored or co-authored over 120 publications. He has been an active investigator in the area of Maternal-Fetal Medicine throughout his career and has been, or is, either a principal or co-investigator for a number of multi-center studies including much of the original work on chorionic villus sampling and multi-fetal pregnancy reductions.

Jingyue Ju, who received his Ph.D. in Bio-organic Chemistry from USC, is Samuel Ruben-Peter G. Viele Professor of Engineering, in the Departments of Chemical Engineering and Pharmacology at Columbia University, where he directs the Center for Genomic Technology & Biomolecular Engineering. With Richard Mathies, he pioneered the use of chemistry and fluorescence energy transfer principles to construct molecular tags for high-throughput genomic protocols, facilitating the development of capillary-array DNA sequencers, which drove the completion of the Human Genome Project. His laboratory invented cleavable fluorescent nucleotide reversible terminators for 4-color DNA sequencing by synthesis, a foundation of next generation DNA sequencers.

Qiao Lin received his Ph.D. in Mechanical Engineering from the California Institute of Technology in 1998 with thesis research in robotics. He conducted postdoctoral research in microelectromechanical systems (MEMS) at the Caltech Micromachining Laboratory from 1998 to 2000, and was an assistant professor of Mechanical Engineering at Carnegie Mellon University from 2000 to 2005. He has been an associate professor of Mechanical Engineering at Columbia University since 2005. His research interests are in designing and creating integrated micro/nanosystems, in particular MEMS and microfluidic systems, for biomedical applications.

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Figure 1.

Principle of bead-based SNP detection. (A) The first cycle of PCR on bead-immobilized reverse primers generates dsDNA. (B) Additional PCR cycles generate dsDNA. (C) A buffer wash purifies bead-bound dsDNA. (D) Template ssDNA is chemically eluted from beads. (E) SBE is performed on bead-bound ssDNA complementary to the template. (F) The SBE product is purified and then desalted for subsequent MALDI-TOF MS detection.

Figure 2.

(A) Schematic of the MEMS-based SNP genotyping device. (B) Cross-sectional view along line a-a illustrating the layered structure of the device. (C) - (G) Fabrication process for the MEMS device: (C) Deposition, patterning and passivation of gold sensor and heater; (D) Fabrication of SU-8 mold; (E) Demolding of PDMS microchamber, and bonding between microchamber and temperature control chip; (F) Deposition of Parylene C; (G) Packing of streptavidin beads.

Figure 3.
Photograph of a fabricated device.

Figure 4.
Experimental setup for MEMS-based SNP genotyping.

Figure 5.
Time-resolved tracking of the chamber temperature.

A

B

Figure 6.

(A) Characterization of bead-based PCR: fluorescent intensity of beads with different PCR parameters, measured in arbitrary units (a.u.). (B) Verification of removal of template ssDNA by NaOH: fluorescent intensity of beads before and after rinsing with NaOH, and after introduction of FAM-modified forward primers. Error bars represent standard deviations based on four independent measurements of fluorescent microbeads.

A

B

c

Figure 7.

Verification of thermal elution and *in-situ* desalting. (A) Fluorescent intensity of beads before desalting, after desalting and after denaturation procedure. (B) Fluorescent intensity of FAM-labeled microbeads following heating. (C) A MALDI-TOF mass spectrum of thermally eluted FAM-modified forward primers. Error bars represent standard deviations based on four independent measurements of fluorescent microbeads.

A

B

Figure 8. MALDI-TOF mass spectrum of SNP detection product with all operations integrated. (A) Mass spectrum of genotyping mutated HBB gene. (B) Mass spectrum of genotyping unmutated HBB gene. (*: extended SBE primer)