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Chemical Gradient-mediated Melting Curve Analysis for Genotyping of Single Nucleotide Polymorphisms

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Abstract

This report describes a microfluidic solid-phase Chemical Gradient-mediated Melting Curve Analysis (CGMCA) method for single nucleotide polymorphism (SNP) analysis. The method is based on allele-specific denaturation to discriminate mismatched (MM) from perfectly matched (PM) DNA duplexes upon exposure to linear chemical gradient. PM and MM DNA duplexes conjugated on beads are captured in a microfluidic gradient generator device designed with dams, keeping the beads trapped perpendicular to a gradient generating channel. Two denaturants, formamide and urea, were tested for their ability to destabilize the DNA duplex by competing with Watson-Crick pairing. Upon exposure to the chemical gradient, rapid denaturing profile was monitored in real time using fluorescence microscopy. The results show that the two duplexes exhibit different kinetics of denaturation profiles, enabling discrimination of MM from PM DNA duplexes to score SNP.

Keywords

chemical gradient; formamide; melting curve analysis; single nucleotide polymorphism; urea

1. INTRODUCTION

Detecting genetic differences between individuals and determining their impact on human health are fundamental in genomic research. As the most common type of human genetic variation, Single Nucleotide Polymorphisms (SNPs) have attracted considerable interest as targets of disease diagnostics [1,2] as well as gene markers [3,4]. SNPs, single base-pair positions in genomic DNA at which different sequence alternatives exist, are estimated to be around 11 million in total [5].

A growing number of new technologies have been developed to type SNPs. A fundamental principal involved in most SNP analysis chemistries is DNA hybridization. Various enzymatic and processing steps typically accompany the hybridization reaction, such as synthesizing with polymerases [e.g. minisequencing [6], pyrosequencing [7] and allele-specific PCR [8]], joining with ligases [e.g. oligonucleotide ligation assay [9]], and nucleic acid-specific cleaving with endonucleases [10], but much can be done with hybridization alone [e.g. allele-specific oligonucleotides hybridization [11]]. Hybridization with allele-specific oligonucleotides (ASO) has been applied on microarray format [12]. However, in its basic form ASO is limited by the difficulties of defining the discriminatory assay conditions

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7. CONFLICT OF INTEREST STATEMENT

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and additional steps, such as enzymatic reactions, have been performed on the oligonucleotide arrays to enhance the discrimination power [13-16]. Furthermore, these methods can be time-consuming, labor-intensive, and costly due to the need for multiple reaction steps, and expensive probes. DNA hybridization can also be tracked in real time by raising the reaction temperature, as in dynamic allele-specific hybridization (DASH) [17]. Although attractive (as no additional enzymes are required), the throughput of the microtiter-plate based DASH method is limited [18].

Miniaturization provides a way to increase throughput at lower cost because small dimensions reduce reagent consumption while enabling an ability to do multiplex analyses. This has made microfluidic technology particularly attractive platform for performing DNA analyses. A number of the SNP technologies described, such as minisequencing [19], oligonucleotide ligation assay [20], and pyrosequencing [21], have been subject to miniaturization and realized in microfluidic formats to meet the needs of increased throughput and reduced costs of reagents and samples. The use of micrometer-scale beads in microfluidic devices for surface-based biochemical assays offers new opportunities, such as increased surface area for improving the analytical capabilities and facilitated liquid handling. Consequently, a wide variety of bead-based microfluidic devices have been reported to type SNPs [22-26]. We have previously reported on a bead-based DASH using monolayers of beads immobilized on chips with integrated heaters and sensors for detecting differences in melting points between PM and MM duplex configurations [27,28]. Although miniaturization to a single bead level was achieved [27], the need for integrated heaters and sensors increases the complexity, and is limited to laboratories with sophisticated microfabrication facilities. Recently, a solution-based DNA analysis assay was described that circumvents the need for heating by using formamide to denature DNA duplexes in a microfluidic device [29]. The present work follows a similar strategy, with the important differences that the discrimination of SNP position located in the center of a target DNA-probe duplex is achieved using solid-phase and real-time melting curve analysis.

Here, we report a simple isothermal solid-phase Chemical Gradient-mediated Melting Curve Analysis (CGMCA) method to discriminate SNP positions - simply by exposing DNA duplexes immobilized on beads to a gradient of formamide or urea generated and precisely controlled by a microfluidic device. Briefly, target DNA immobilized on beads, annealed to an allele-specific probe with the variant base located in the center, is captured perpendicular to a flow-through gradient generating channel. The DNA duplexes are exposed to linear chemical gradient generated upstream and the denaturation process is monitored in real time. Additionally, we report on a device with two parallel cross-channels perpendicular flow for pair-wise comparison to score the presence of a SNP position in a single run.

2. MATERIAL AND METHODS

2.1 Target DNA and probe

The target DNA oligonucleotides and probes used in this study were as follows: The allele-specific probe: 5'-Cy3-ACACAAAC**G**CTGTTGGA -3'; The matching target-DNA: 5'-biotin-ATGAGAGAAGAGAGACTATTTTCCAACAGCGTTTGTGTTTCTTGGATGTCATTCT-3'; Mismatching target-DNA: 5'-biotin-ATGAGAGAAGAGAGACTATTTTCCAACAGGGTTTGTGTTTCTTGGATGTCATTCT-3'. The SNP positions (C/G) are indicated in bold. The oligonucleotides and probes were purchased from IDT (Coralville, IA, USA). The mutation was placed in the middle of the sequence to increase the destabilizing effect of the duplexes.

2.2 Microfluidic device fabrication

The microfluidic device was fabricated by casting polydimethylsiloxane (PDMS; Dow Corning, Midland, MI) polymer on a resist-structured silicon wafer according to standard soft lithographic techniques. A 2-layer SU-8 master on a silicon wafer with structures in SU-8 resist (MicroChem, Newton, MA, USA) was produced according to the supplier's recipe using standard MEMS technology. The PDMS was mixed (10:1, w/w) with a cross linker, poured on top of the silicon wafer, degassed and cured at 65° C for 6 hours. The PDMS with the replicated channels was peeled off from the master, and channel access holes were punched with a 22-gauge needle. The PDMS replica was bonded to a glass slide via oxygen plasma. Access tubing (Tygon; Small parts, Miami Lakes, FL, USA) of slightly larger diameter, was press-fitted into the holes.

2.3 Sample Preparation

The single stranded target oligonucleotides were immobilized on nonmagnetic streptavidin-coated 10 μ m beads (Bangs Laboratories Inc., IN, USA) by incubating 1 μ L of 5 μ M oligonucleotide with 20 μ L of beads and 30 μ L of binding buffer (10 mM Tris-HCl, 2 M NaCl, 1 mM EDTA, 0.1% Tween 10) for 30 min with mixing. After a washing step with 10 mM Tris-acetate, pH 7.6, the beads bearing the immobilized oligonucleotides were resuspended in 30 μ L of annealing buffer (100 mM Tris-acetate, pH 7.6, 50 mM magnesium acetate), and 1 μ L (5 μ M) of the allele-specific probe was added. Hybridization was performed by incubating at 85 °C for 2 min and then cooling to room temperature. The DNA duplexes immobilized on streptavidin-coated beads were washed 3 times with 10 mM Tris-acetate, pH 7.6, solution and finally resuspended in 50 μ L deionized water.

2.4 Isothermal Melting Curve Analysis

The device was first primed by filling with deionized water and inlet 1 (deionized water) and 2 (chemical solution) reservoirs were connected without trapping air bubbles. The tube at inlet 2, containing the chemical solution, was initially clamped such that the entire channel system of the device was filled only with water and released first after the beads were trapped and ready to initiate the reaction. In addition, the water reservoir (inlet 1) was kept on a higher level initially. A pipette containing 10 – 20 μ L bead solution is attached to the bead-inlet (by “dispensing” the pipette inside the inlet whole of the PDMS device) and suction is applied from the other side of the channel to fill the beads. Once the channel, designed with dams to keep the beads isolated from the perpendicular gradient generating channel, is filled with beads, the pipette is removed and the inlet is sealed during the remaining process. The chip was placed under an inverted fluorescent microscope and the reaction initiated by applying suction (1 μ L/min) from the main outlet, and the water reservoir is lowered to the same level as the chemical reservoir. Pictures were taken every ten seconds and a shutter closing between each picture was used to prevent bleaching of the fluorescent sample. Images were collected and further analysis was conducted using ImageJ (<http://rsb.info.nih.gov/ij/>).

3. RESULTS

We introduce a microfluidic solid-phase Chemical Gradient-mediated Melting Curve Analysis (CGMCA) method to denature DNA duplexes. For this purpose, we have built a microfluidic device that is capable of capturing and exposing DNA duplexes to a precisely controlled chemical gradient of formamide and urea. We applied solid-phase CGMCA to discriminate single-base MM from PM DNA duplexes.

3.1 Principle of CGMCA and device characterization

Chemical Gradient-mediated Melting Curve Analysis exploits the fact that even a single mismatch between probe and the target DNA will significantly reduce the melting temperature of double stranded DNA. Thus, analog to heating, target DNA- probe duplexes containing destabilizing mismatches denature at lower chemical concentration than probes bound to a perfectly matched target DNA. An overview of the principle of CGMCA is shown in Figure 1a. A biotinylated target DNA sequence is immobilized on a streptavidin-coated bead. A fluorescently labeled oligonucleotide probe, specific for one allele, is hybridized to the target DNA. The sample is then trapped in a gradient generating microfluidic device and exposed to a linearly increasing chemical gradient perpendicular to the flow direction while the fluorescence is continually monitored. A rapid fall in fluorescence indicates the denaturing (or “melting”) of the probe-target duplex. The presence of a single-base mismatch at the center position between the probe and the target results in a lowering of the chemical concentration required to denature DNA duplexes and is detected using a fluorescence microscope.

The microfluidic linear gradient generating device was designed and fabricated by standard soft lithography techniques using poly(dimethylsiloxane) (PDMS). An overview of the PDMS device is shown in Figure 1b. The device was designed for dual purpose: to generate linear gradient and to capture DNA duplexes perpendicular to the gradient channel. The linear gradient is generated by diffusive mixing of solutions flowing at laminar flow inside a network of microchannels [30]. Water and a chemical solution are introduced from reservoirs connected to inlet 1 and 2 and are repeatedly combined, mixed, and split inside the channel network to yield distinct mixtures with distinct compositions in each of the branch channel. Once the branch channels are recombined, the gradient is flown through a cross-channel with trapped beads perpendicular to the flow direction. Devices with a single bead trapping channel (inset, Figure 1b) and two parallel bead trapping channels as illustrated in Figure 1b were evaluated. The height of the gradient generating and beads trapping channels were 25 μm . These channels were connected by 5 μm height dams on each side of the bead trapping channel, effectively trapping the 10 μm beads while allowing the fluid through. The addition of the cross-channel allows capturing and interrogation of solid-bound DNA without disturbing the gradient generated upstream in the channel network.

The precise initiation of the chemical gradient reactions on the device is controlled from one outlet. Initially, reservoir 1 (water connected to inlet 1) is kept at a higher level compared to reservoir 2 (chemical solution connected to inlet 2), allowing only water to flow inside the network of microchannels when negative pressure is applied from the outlet. To initiate the controlled denaturation reaction, reservoir 1 is lowered to the same level as reservoir 2 leading to balanced flow conditions and the linear gradient is initiated. This procedure prevents unintended exposure of high concentration chemical solution to the captured DNA duplexes without the requirement of integrated valves or other equipment to control the fluid flow.

We first characterized the concentration profiles using water and fluorescein isothiocyanate dye as starting solutions. The formation of the linear gradient starts with controlled mixing of the two solutions by applying negative pressure from the outlet. Details of the fluorescence distribution across the bead trapping cross-channel are shown (Figure 1c, top panel), together with the measured fluorescence profiles (Figure 1c, bottom panel). The streamlines of the gradient are notably parallel to the fluid flow direction, indicating that the linear gradient generated is maintained after crossing the bead trapping cross-channel.

3.2 DNA denaturing profile

To determine the DNA denaturing profile over chemical concentrations and exposure time, we prepared 10 μ m beads bound to MM DNA-probe duplex with a single-base mismatching in the center of the probe sequence. We prepared two buffer solutions, one containing 50% formamide and another contained 8M urea dissolved in water. The beads were trapped inside the device and exposed to the chemical gradient (Figure 2). At low formamide and urea concentrations the DNA-probe duplexes are intact, seen as strong fluorescence (Figure 2a). At high formamide and urea concentrations the duplexes are dissociated into single strands, leading to a rapid fall in fluorescence. We monitored the denaturing profile by taking picture every 10 seconds for 15 minutes (Figure 2b-c). An average intensity of the area covering each channel, corresponding to respective chemical concentration, was tracked in real time. In the case of formamide (Figure 2b), a rapid fall in fluorescence corresponding to the higher concentrations is observed. At 0% formamide concentration, the fluorescence profile is constant while for concentrations above 37% rapid denaturation is obtained within minutes. A clear denaturing profile appears within 5 minutes.

In the case of urea (Figure 2c), the initial base-line fluorescence is lowered upon exposure to urea. This is observed for all concentrations, except for the first channel corresponding to only water. Apparently, the viscous urea solution absorbs some of the fluorescence intensity. From the new base-line, however, a denaturation profile similar to the one with formamide is observed. Using the device, we were able to obtain the concentration needed to denature the duplexes as well as the dynamics of the denaturation process for each concentration in time.

3.3 SNP calling

To determine the ability of the method to discriminate a single base mismatch, we prepared PM and MM DNA duplexes on beads. For each denaturant, two devices were prepared (one for PM and another for MM duplexes). The duplexes were exposed to the gradient and observed under an inverted microscope after 15 minutes. Allele-specific discrimination is achieved by the difference in the denaturing profile between the probe set and match or mismatch target (Figure 3). As can be seen in Figure 3a, the mismatching allele generated a drop in fluorescence at a lower formamide concentration while the matching generates a fluorescence intensity drop at a higher concentration. The heterozygous sample, comprising 1:1 ratio of the match and mismatch duplexes, produced a curve that is the sum of the two homozygous curves, in agreement with previous reported temperature based melting curve analysis [28]. In the case of urea, the heterozygous sample could not be resolved accurately due to the base-line reduction of the signal intensity. However, when the intensity signals were normalized for 1M urea (Figure 3b), the homozygous PM and MM samples could be differentiated accurately. While it requires higher than 8M urea concentration to denature the PM DNA-probe duplexes the MM duplexes are denatured at concentrations higher than 4M. In both the formamide and urea cases, the large concentration differences for denaturing MM and PM duplexes assures single-base discrimination.

3.4 Parallel analysis

Device with two parallel cross-channels perpendicular to the flow was designed and evaluated for pair-wise comparison to score the presence of a SNP position in a single run. Figure 4 shows beads conjugated with cy-3-labeled DNA duplexes trapped in the device before exposure to formamide and after 15 minutes of exposure. As can be seen from the fluorescence intensity profile in Figure 4b, the MM DNA duplex is denatured at a lower formamide concentration compared to the PM DNA duplex. The DNA denaturing profile obtained here is similar to the one obtained using two different single-cross-channel devices (from Figure 3), showing repeatability of the denaturing profile and that the gradient

streamline through the two parallel bead-trapping channels is parallel to the flow direction. The difference between the MM and PM duplexes denaturation curves displays easily interpretable peaks at the melting point when the negative derivative versus concentration is plotted (Figure 4d). As can be seen in Figure 4d, the MM sample has a distinct peak at formamide concentration of 28%, indicating the denaturing concentration, while the PM sample has a peak at 40% formamide concentration. The large difference between the two denaturing points enables SNP calling. Additionally, the pair-wise analysis enables the analysis in a single run with added internal control because the two alleles are analyzed under the same experimental conditions.

The results presented in Figure 4 are end-point analysis after 15 minutes of reaction. It is also possible to obtain a quantitative estimate of the discrimination power of the assay within minutes by comparing the dissociation kinetics alone. The discriminatory power of the assay can be measured by taking the ratio between the intensity signals of the PM and MM samples along the concentration gradient. Figure 5a shows the ratio (PM intensity/ MM intensity) plotted against time. Maximum ratio is obtained for the highest formamide concentration, 50%, within 2 minutes. After reaching a peak, a rapid decrease in the ratio indicates that the PM duplexes are catching up in the dissociation process. A maximum ratio value of 22 was obtained, indicating significantly different kinetics to effectively discriminate the MM over the PM sample. Figure 5b shows the denaturing profile after 2 minutes, confirming the significant difference in kinetics between the two alleles.

4. DISCUSSION

Recent development in microfluidic technology has enabled many of existing SNP genotyping methods to be adapted in microchip systems. Microfluidic devices are an attractive approach, because the integration of active fluidics in a microenvironment overcomes several of the limitations of conventional and microarray systems, offering many distinct benefits including the ability to manipulate exceptionally small volumes of liquid, resulting in low DNA sample and reagent consumption, short analysis times, and improved sensitivity. In addition, these systems can be mass-fabricated at low cost, especially when polymer-based materials are used in their construction. More important, microfluidic systems enable processes that are not possible on a macroscale, such as generating precisely controlled gradient of chemical solutions applied in this study.

Monitoring the thermal transition between double stranded DNA and single stranded DNA is the principle tool used in many assays [17,31,32]. Although measuring DNA melting curves is essential for these techniques, current methods are hindered by the need to ramp the temperature dynamically combined with real time detection. We describe the combination of on-chip DNA capturing with an on-chip chemical gradient to produce a stable melting curve for DNA duplexes. Allele-specific hybridization is enhanced by tracking the denaturation of DNA duplexes chemically instead of dynamic heating. The simple microfluidic device was able to capture beads without cross-talk or disruption of the generated gradient. This is critical because the method relies on stable gradient to discriminate a single base mismatch. Negative pressure applied from the outlet controls the fluid flow inside the microfluidic network. This has enabled us to keep the device design and overall experimental procedure simple. Due to the flexible nature of the PDMS material, the beads were easily loaded by leaving a pipette tip with precise volume of beads at the inlet whole of the bead trapping channel. Applying negative pressure from the other side of the bead trapping channel enabled the controlled filling of the beads without trapping air bubbles. The entire time for priming the device and filling the beads took about 5 minutes, keeping the total analysis time to 20 minutes.

Formamide does not chemically degrade nucleic acids at room temperature and is therefore frequently used to lower the DNA melting temperature in PCR reactions to avoid DNA degradation. The reported values for lowering of the melting temperature of DNA duplexes per percent formamide in solution range from 0.60 to 0.72°C [33,34]. Formamide was recently used in a solution-based microfluidic assay for DNA analysis [29]. However, solution-based assays are inherently limited for multiplexing capabilities and parallelization as the DNA needs to be mixed with a buffer prior to introduction to the device. In addition, a more complicated detection system is used to avoid background fluorescence from the dissociated DNA. In the present work, the background is avoided as the dissociated DNA probe is constantly removed and the use of microbeads as a solid phase enables parallelization. Urea is another denaturant with properties widely exploited in diverse biochemistry protocols and is commonly used in conjunction with polyacrylamide for the electrophoretic fractionation of basic proteins [35]. Prior to the introduction of formamide, urea was commonly applied to nucleic acid hybridization [36,37]. Although urea is known for the ability to destabilize the DNA duplex, to our knowledge urea has not been used in DNA analysis assays before.

Urea is highly soluble in water and the corresponding solution has shown to have nonlinear optical properties [38]. This can probably explain the reduction in intensity for all but the first channel containing only water solution. Consequently, urea-based denaturing could not resolve heterozygous samples. Although more experiments will be required to fully characterize the optical property of urea-solution for the purpose of DNA analysis, the baseline reduction in intensity seems to be constant for the range of concentrations (1-8M) used in our experiments. Contrary to urea, the formamide based CGMCA scored all possible (three) variants of the SNP, something that has not been shown before. A rapid denaturing profile is obtained within minutes of exposure of the DNA duplexes. Finally, it is worthwhile to note that formamide is a recognized hazardous solvent with potential toxicity. This organic solvent has a relatively low boiling point (210 °C), which results in substantial evaporation at the high experimental temperatures associated with PCR reactions. Here, the solvent is used at room temperature and at a fraction of the volume. For instance, only 5 µL of 50% formamide solution is used for a 10 minutes reaction experiment and the closed nature of the microfluidic network prevent direct contact with the solvent. On the other hand, urea is a none-toxic alternative readily available in every laboratory.

Unlike direct DNA sequencing, such as Sanger's or Pyrosequencing, majority of SNP methods are based on comparison between the two alleles to confirm the presence of a SNP position. Often, the experimental conditions must be optimized in order to selectively increase destabilization effects of mutation on mismatched duplexes and particular attention must be paid to hybridization and rinsing conditions (temperature, buffer stringency,...) as well as probe design (length, position of the mutation, GC/AT content, ...). For each new mutation, another optimization work must be achieved to find the best condition that allows for the detection of the mutation. Since a range of chemical concentration is scanned, a prior knowledge of the dissociation kinetic is not necessary and thus no previous optimization is needed. Analogy to temperature based systems can be made by monitoring the first negative derivative of the denaturing profile. A peak indicates the melting temperature (T_m), which is defined as the temperature at which 50% of the DNA becomes single stranded. Allele-specific discrimination is demonstrated by the difference in melting concentration (a peak analog to T_m) between the probe set and match or mismatch template (Figure 4d). The differences in melting concentration for the MM and PM samples were 12%, which ensures unambiguous discrimination. Additional power of the method presented here is the use of initial dissociation kinetics as a means to differentiate target DNA-probe configurations for rapid SNP calling and the ability to parallelize analysis to reduce time and cost.

5. CONCLUSIONS

In summary, we report on a microfluidic chemical gradient-mediated method capable of discriminating single-base MM duplexes over PM. The simple microfluidic device was able to capture and expose DNA duplexes immobilized on beads to a stable chemical gradient. As shown by the different kinetics as well as end-point analysis of the denaturation process, allele-specific discrimination was successfully demonstrated. Using formamide as denaturant, we showed that it is possible to score all three variants of the SNP position. This simple method involves standard run conditions, requires no enzymes or heating to discriminate single base mismatched DNA duplexes, and is easily scalable for rapid high throughput DNA analysis. Further improvements are necessary to circumvent the non-linear optical properties of urea. Future work consists of critically evaluation of the formamide method to detect point mutations in biological PCR samples.

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Figure 1.

(a) Principle of solid-phase CGMCA: The target DNA is conjugated on beads and fluorescently labeled allele-specific probe is annealed using standard protocol. The beads are captured inside the microfluidic device and exposed to a linear chemical gradient. The fluorescent intensity is monitored in real time by an inverted microscope: a rapid drop in fluorescence is visualized as the denaturing point of the duplexes. (b) Microfluidic gradient generator device: The gradient generating network channels and bead trapping channels are 25 μm in height, connected by 5 μm dam filters to effectively trap 10 μm beads perpendicular to the gradient flow. The diffusion-based linear gradient is generated by continuously combining and branching of water from inlet one and chemical (formamide or urea) solution from inlet two. The inset shows a single bead-trapping channel packed with beads perpendicular to the flow direction of the chemical gradient (Scale bar: 75 μm). The gradient, branched into 9 channels with known concentrations is combined at the interface of the bead trapping channel.. (c) Evaluation of linear gradient: Upper panel shows fluorescent image of a gradient generated by mixing water with a fluoresceine dye solution. The area of the gradient generating channels (i) just before merging to the bead-trapping cross-channel (ii) and after crossing the channel (iii) is highlighted (Scale bar: 50 μm). The lower panel shows cross-section intensity of the three areas (i, ii and iii). Close to identical intensity profile was obtained for all three areas, indicating that the gradient is not disturbed by the cross-channel.

Figure 2.

DNA denaturing profile. (a) Fluorescence image of MM DNA duplexes, before (i) and after 15 minutes of exposure to a linear gradient of formamide (ii) and urea (iii) solution. The linear gradient increases from left (solution containing 0% chemical) to right and the highlighted dotted boxes correspond to each of the 9 channels with specific concentration of the chemical solution. Scale bar: 100 μ m. (b) Formamide-mediated denaturing profile of the MM DNA. The average intensity of each dotted boxes corresponding to the specific formamide concentration is plotted against time. A rapid denaturing kinetic is observed for the higher concentrations while the lower concentrations have slow kinetics. The denaturing profile reaches equilibrium and is more or less constant after 10 minutes of exposure to formamide solution. (c) Urea-mediated denaturing profile of the MM DNA. As the urea solution start to flow over the trapped beads, notable intensity reduction is observed for all concentrations but the first channel containing no urea. From this new base-line, a gradual increasing denaturing kinetic is observed for urea concentrations of 4 M and above while the lower concentrations remain constant.

Figure 3. SNP calling based on chemical gradient-mediated melting curve analysis using formamide (a) and urea (b) as denaturants. The reaction was allowed to run for 15 minutes in a single cross-channel device and the normalized signal intensity was plotted against the chemical concentration. A rapid fall in intensity indicates the denaturing concentration. The urea-based samples (b) were normalized at 1M concentration to compensate reduction in intensity signal due to the solution.

Figure 4.

Pair-wise SNP analysis. (a) A bright field image of the two parallel cross-flow channel device filled with beads conjugated with DNA. The upper channel was packed with MM and the lower with PM DNA duplexes. (b) Fluorescent image of the DNA denaturing profile after 15 minutes of exposure to formamide gradient. The MM DNA duplex has lower denaturation concentration. Scale bar: 100 μm . (c) Resulting fluorescent intensity change versus formamide concentration. (d) Negative derivative of the intensity signals. The peaks represent the melting concentrations of the MM and PM DNA samples. A difference of 12% in formamide concentration between the peaks (28 % and 40% formamide concentrations for respective peak) is obtained, indicative of the discrimination power of the assay.

Figure 5.

Dissociation kinetics of the pair-wise analysis. (a) The PM to MM ratio (PM intensity/ MM intensity) of intensity signals along the concentration gradient versus time is plotted. The maximum ratio is obtained for the 50% formamide concentration within 2 minutes of experiment time. (b) Resulting fluorescent intensity change versus formamide concentration at which maximum ratio between PM and MM intensity is obtained.