Solvent resistant microfluidic DNA synthesizer†

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We fabricated a microfluidic DNA synthesizer out of perfluoropolyether (PFPE), an elastomer with excellent chemical compatibility which makes it possible to perform organic chemical reactions, and synthesized 20-mer oligonucleotides on chip.

Synthetic chemistry presents numerous instances in which it would be useful to automate and miniaturize reactions. Many synthesis problems require trial and error effort in order to optimize yield and a miniaturized automated chemical synthesizer would reduce manpower, reagent consumption, and cost. There are other cases, such as DNA synthesis, where the process has been optimized over decades of work and one desires to take advantage of these efficient reaction chemistries. Reducing reagent consumption for chemical synthesis also offers the possibility of reducing waste proportionately and is thus environmentally friendly.

Microfluidic devices have shown great potential towards realizing this goal.[1] However, because chemical synthesis requires the use of a wide variety of solvents, most microfluidic work in this area has been limited to continuous flow reactors fabricated in glass or silicon, which limits the workable complexity and reagent savings.[2,3] In special cases where the solvents are mild, it has been possible to use integrated elastomeric micromechanical valves to perform batch synthesis at the nanogram scale of radiopharmaceuticals[4] and 'click-chemistry' protein ligands.[5] Hua *et al.* have attempted DNA synthesis in a hybrid silicon–elastomer microfluidic valve system, but with limited chemical characterization of the products.[6] We previously reported a photocurable perfluoropolyether (PFPE) elastomer, which is compatible with a wide variety of solvents[7] and is a suitable material with which to fabricate micromechanical valves.[8]

Here we report a microfluidic DNA oligonucleotide synthesizer made of PFPE which performs reaction cycles adopted from the widely used phosphoramidite method.[9,10] The synthetic procedure is shown in Scheme 1. The device (Fig. 1) is capable of synthesizing 60 pmol of DNA oligonucleotides while consuming less than 500 nL of 0.1 mol L^{-1} phosphoramidite solution in each reaction cycle. The reduction of reagent consumption is significant: a 60 fold reduction over conventional automation. This approach demonstrates the usefulness of integrated micromechanical valves for complicated multi-step organic synthetic reactions and enables automated chemical experiments with a wide variety of solvents.

PFPE was synthesized according to ref. 7. Microfluidic molds were prepared using standard photolithographic methods. The uncured PFPE was bubbled with oxygen-free nitrogen before UV exposure and then poured or spin-coated onto the molds to form layers with different thicknesses. The three layered architecture of the synthesizer is based on valve structures used in other elastomeric devices.[11,12] The layers were partially cured by UV light and then bonded together with further UV exposure. All of the PFPE fabrication steps were carried out under nitrogen atmosphere inside a glove-box.

The first nucleotide (dT in our experiments) was pre-attached to the porous silica beads (pore size 200 nm, Sepax Technologies, DE, USA) with a base-cleavable succinyl linker at the 3′-end. The 5′-position was protected with a dimethoxytrityl (DMT) group. The cross-section of the column is 300 × 15 μm, and the typical length of the column is 4 mm. All the reaction reagents were delivered through Teflon tubes from vials pressurized by argon (10 psi). The column valves were actuated with 40 psi pressure to prevent the beads from escaping.

We used 3% dichloroacetic acid in dichloromethane (DCM) as the deblocking reagent. DCM is a solvent that poses severe challenges with conventional elastomers such as polydimethyl siloxane (PDMS).[13] Microfluidic channels made in PDMS will swell and become clogged within seconds when exposed to DCM, but PFPE is resistant to DCM and the chip is fully functional for hours.

We substituted iodine with (1*S*)-(+)-(10-camphorsulfonyl)oxaziridine (CSO),[14] as the oxidizing reagent because in our experiments CSO led to purer target products. The coupling step requires two reagents, phosphoramidite and 5-ethylthio-1H-tetrazole as activators. The two reagents were piped into the synthetic column in alternate fashion. The total time of each synthetic cycle is 9 min, including 2 min of deblocking, 2 min of coupling, 2 min of oxidizing and three washing steps, 1 min each.

We synthesized 20-mer DNA oligonucleotides with the sequence: 5′-CCG ACC TGG ATA CTG GCA TT-3′. After the last cycle, we used deblocking solution to deprotect the DMT group at the 5′-end. Finally the beads were washed using acetonitrile before they were flushed out of the column into a micro-vial. The beads were then lyophilized and cleaved using concentrated ammonium hydroxide for 1 h. The resulting solution was collected and kept at room temperature for 4 h to completely remove the side-protecting group of each base. The solution was lyophilized again to eliminate residual NH_3 and water. We re-suspended the synthesized oligonucleotide samples into

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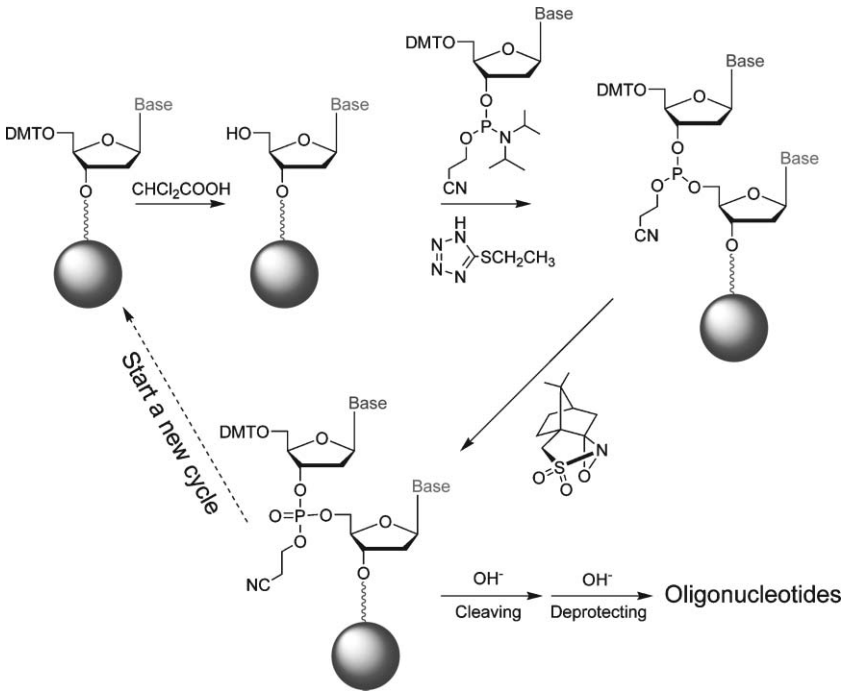

Scheme 1 DNA synthesis procedure.

Tris-EDTA buffer (pH 7.5) to carry out HPLC/MS detection and electrophoresis, without further purification. Electrospray ionization (ESI) MS confirms the molecular weight of 6092.5 (calculated: 6093) of the final product and shows a yield of 67.7%, with most of the impurities coming from single deletions (23%) and single insertions (4%). The gel image (Fig. 2(B)) also shows that the synthesized product had the same mobility as the commercially ordered HPLC-purified sample with the same sequence.

To further test that our synthesized oligonucleotide had the correct sequence, we measured the melting curve of the sample with complementary strands and oligonucleotides containing a single-base mismatch. Our synthesized DNA was labeled at the 5′-end with Cy3-phosphoramidite. The two HPLC purified strands, complementary and single-mismatched, are purchased, with carboxyfluorescein-labels (FAM) at the 3′-ends.

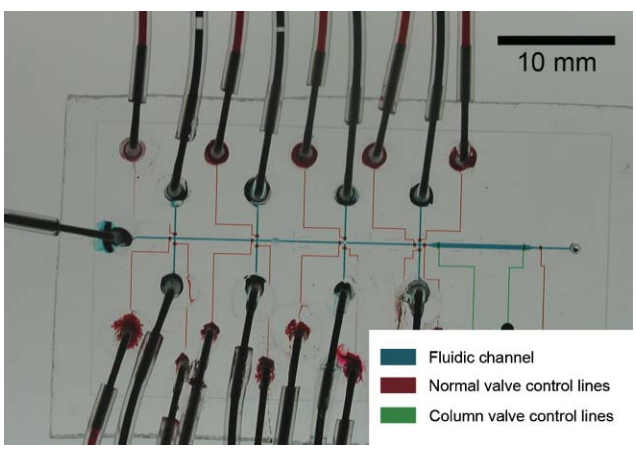

Fig. 1 Optical micrograph of the PFPE DNA synthesizer chip. The channels have been filled with food dyes to indicate the different functional parts of the chip.

When the two strands are hybridized, the fluorescent intensity of FAM is significantly reduced through FRET interaction between FAM and Cy3 fluorophores. When the temperature exceeds the melting temperature, the two strands separate and the FAM signal recovers. By monitoring the fluorescent intensity of the FAM *versus* temperature, we measured the melting curve of the

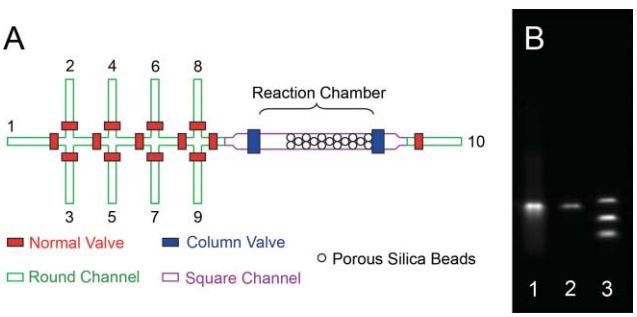

Fig. 2 Schematic of microfluidic oligonucleotides synthesizer. (A) The fluidic channels consist of both rounded and squared profiles. The first eight channels are assigned to specific reagents: (1) acetonitrile, (2) deblocking reagent, (3) oxidizing reagent, (4) activator, (5) dT-CE phosphoramidite, (6) Pac-dA-CE phosphoramidite, (7) iPr-Pac-dG-CE phosphoramidite, and (8) Ac-dC-CE phosphoramidite. The ninth channel serves two functions. During experimental setup it is used as an inlet for silica beads. During the experiment it is used as an outlet for unwanted reagents that are left in the main channel. A solid-phase reaction column is formed *in situ*[4] using partially closed column valves to trap the silica beads (5 μm in diameters). (B) Gel image of (1) oligonucleotides synthesized using our microfluidic device, (2) HPLC-purified oligonucleotides standard purchased from IDT, and (3) HPLC-purified $(dT)_{10}$-$(dT)_{15}$-$(dT)_{20}$ oligonucleotide size standard purchased from IDT. The motility of the synthesized product is identical to that of commercially ordered HPLC-purified sample. However, trace byproduct can be seen in the unpurified sample.

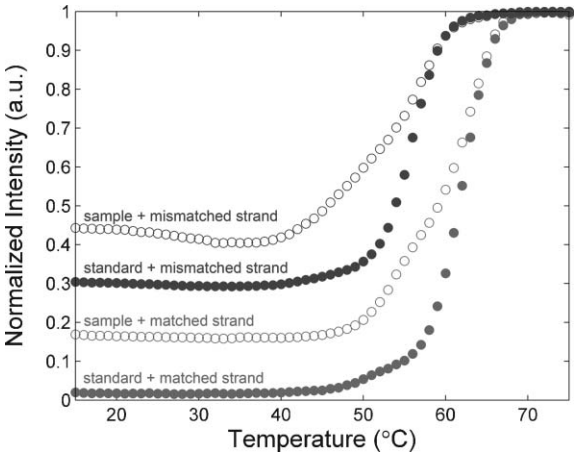

Fig. 3 Melting curve measurements of single strand (ss)-DNA 20-mer with complementary strand and oligonucleotides with a single mismatch, respectively.

oligonucleotides (Fig. 3). The measurement was carried on a commercial microfluidic chip (Digital Isolation and Detection Chip, Fluidigm, South San Francisco, CA) which takes 12 parallel measurements at a time and each measurement is performed with 1200 replicates. Compared with the standard samples, our synthesized oligonucleotides show similar melting temperature but higher fluorescent intensity at lower temperatures, indicating that there is faint impurity in the product. This observation coincides with the results of electrophoresis and mass spectrometry.

In conclusion, we have demonstrated that microfluidics can be used for batch synthesis of DNA using phosphoramidite reagents and conventional solvents. We envision a number of direct applications, in spite of the fact that only picomoles of product are produced. First, DNA is special in that it can be biochemically amplified and it has been shown that one can perform whole gene synthesis with as little as femtomoles of the source oligonucleotides.[15] Second, if the assay is to be performed on the chip, it is possible to elute the product DNA in nanolitre volumes, thus creating concentrations that are comparable to what are used at the benchtop. This may be useful, for example, in screening siRNA sequences,[16] creating DNA nanostructures,[17] and for DNA computing.[18,19]

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Supplementary Material (ESI) for Lab on a Chip

Solvent Resistant Microfluidic DNA Synthesizer

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I. Experimental

1. Materials

Porous silica beads (5 μm in diameters, pore size 200 nm) were purchased from Sepax Technology (Newark, DE, USA). Fluorolink D4000 was purchased from Solvay Solexis (Thorofare, NJ, USA). Dichloropentafluoropropane was purchased from SynQuest Laboratories (Alachua, FL, USA). Isocyanatoethyl methacrylate (EIM, 95%) was purchase from Monomer-Polymer & Dajac Labs (Feasterville, PA, USA). 3-Aminopropyl-trimethoxysilane (APTMS), N,N-diisopropylethylamine (DIEA), (1S)-(+)-(10-camphorsulfonyl)oxaziridine (CSO), anhydrous toluene, 2,2-Dimethoxy-2-phenyl acetophenone (DMPA, 99%), and Dibutyltin diacetate (DBTDA, 99%) were purchased

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from Aldrich (Milwaukee, WI, USA). 5-Ethylthio-1H-tetrazole (activator, 0.25 mol/L solution in anhydrous acetonitrile), deblocking mix (3% dichloroacetic acid (DCA) in dichloromethane (DCM)), anhydrous acetonitrile (MeCN), 5'-dimethoxytrityl-N-phenoxyacetyl-2'-deoxyAdenosine-3'-[(2-cyanoethyl)-(n,N-diisopropyl)]-phosphoramidite (Pac-dA-CE phosphoramidite), 5'-dimethoxytrityl-N-acetyl-2'-deoxyCytidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Ac-dC-CE phosphoramidite), 5'-dimethoxytrityl-N-p-isopropyl-phenoxyacetyl-deoxyGuanosine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (iPr-Pac-dG-CE phosphoramidite), 5'-dimethoxytrityl-2'-deoxyThymidine-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (dT-CE phosphoramidite), 1-[3-(4-monomethoxytrityloxy)propyl]-1'-[3-[(2-cyanoethyl)-(N,N-diisopropyl)phosphoramidityl]propyl]-3,3,3',3'-tetramethylindocarbocyanine chloride (Cy3-phosphoramidite) were purchased from Glen Research (Sterling, VA, USA). 5'-O-(4,4'-dimethoxytrityl)-thymidine-3'-O-succinic acid (5'O-DMT-2'-dT-3'-O-succinate) was purchased from Monomer Science, Inc. (New Market, AL). 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) was purchased from Anaspec (San Jose, CA, USA). SYBR Gold nucleic acid gel stain, pre-cast 15% TBE-Urea denaturing polyacrylamide gels (1.0 mm, 10 well), and ultrapure water were purchased form Invitrogen (Carlsbad, CA, USA). Tris-EDTA (TE) buffer (pH 7.5) was purchased from Integrated DNA Technologies (IDT, Coralville, IA, USA). All the DNA oligonucleotides, except the ones we synthesized from the microfluidic chips, were ordered from IDT and HPLC purified.

2. Synthesis of Perfluoropolyethers (PFPEs)

In a typical synthesis, Fluorolink D4000 (1000 g, 0.24 mol) was added to a dry 2 L round bottom flask and purged with argon for 15 minutes. EIM (67.56 mL, 0.48 mmol) was then added via syringe along with Dichloropentafluoropentane (319 mL), and DBTDA (8 mL). The solution was immersed in an oil bath and stirred at 50^{o}C for 24h. The solution was then passed through a chromatographic column (silica, Dichloropentafluoropentane, 5 × 5 cm). Evaporation of the solvent yielded clear, colorless, viscous oil (PFPE DMA) which was further purified by passage through a 0.22 μm polyethersulfone filter.

In a typical cure, 1 wt% or 0.1% wt of DMPA (0.5 g or 0.05 g, 20.0 mmol or 2 mmol) was added to PFPE DMA (50 g, 12.0 mmol) along with 20 mL Dichloropentafluoropentane until a clear solution was formed. After removal of solvent, the viscous oil was passed through a 0.22 μm polyethersulfone filter to remove any DMPA that did not disperse into the PFPE DMA. The filtered PFPE DMA was then irradiated with a UV source (Electrolite UV curing chamber model no. 81432-ELC-500, λ = 365nm, with only one of the four lamps operating to produce 7 mW/cm^2 power flux) while under an oxygen-free nitrogen purge. The fully cured PFPE has tensile modulus and oxygen permeability (3.9 MPa , 400 barrers) similar to that of PDMS (2.4 MPa, 550 barrers).

3. Fabrication of the Microfluidic Chips

All the fabrication steps were conducted in a glove box purged with N_2. The PFPE DMA was prepared with 2 different concentrations of photoinitiator, 0.1% and 1.0%. Before use, the polymer was bubbled with N_2 for 30 min. A thick layer (2 mm) of PFPE

DMA containing 0.1% of photoinitiator was poured onto the Si wafer having the desired flow pattern made of AZ50 (AZ Electronic materials) and SU-8 2025 features (only the reaction chamber for trapping beads). The thick layer was irradiated with a UV source (Electrolite UV curing chamber, ELC-500, λ = 365nm, 7 mW/cm^2) for 12 sec. Then PFPE DMA with 1.0% of photoinitiator was spin coated to a thickness of 30 μm (800 rpm for 20 s) onto a Si wafer with the desired control pattern made of AZ50. This wafer was placed in the UV chamber and irradiated for 5 sec. The thick layer was removed, aligned to the thin layer and then irradiated for another 24 sec to bond them together. The chip was peeled off and took out of the glove box to punch inlet and outlet holes in the fluidic layer as well as the control layer; then it was placed back into the glove box. A 40 μm thick layer of PFPE DMA with 1.0% photoinitiator was spin coated (600 rpm 20 s) onto a glass slide and then irradiated for 4 sec. The chip was placed on top of the coated glass slide and then irradiated for 5 min.

4. Operation of the Microfluidic Chips

All the inlets and outlets of the chips are inserted with steel tubes (New England Small Tube Co., Litchfield, NH) and connected with either microbore PTFE tubing (for chemical reagents) or Tygon tubing (for control valves). All the tubing are purchased from Cole-Parmer (Vernon Hills, IL). The valves are filled with Krytox oil. Both reagent delivery (10 psi) and valve actuation (30 – 40 psi) are pressured by argon. The pressure is switched on and off by computer-controlled solenoid valves (Pneumadyne, Plymouth, MN). Chips were operated on a single-use basis.

5. Synthesis of the Oligonucleotides

The porous silica beads were modified with the first nucleotide (Thymidine in our experiments) attached. We added 0.5 g silica beads into 10 mL 8 mol/L HNO_3, and refluxed it for 4 hours. The beads were washed with water and then dried in an oven at 120 °C overnight. The dried beads were re-suspended into 10 mL anhydrous toluene and then 0.6 mL APTMS was added into the system. The mixture was refluxed for 24 hours and then filtered and washed with toluene and acetone. All beads were then transferred into a new vial with 75 mg 5'O-DMT-2'-dT-3'-O-succinate, 40 mg HATU, 100 μL DIEA, and 5 mL MeCN. The reaction vial was sealed and the mixture was stirred overnight at room temperature. Finally the beads were filtered, washed with MeCN, acetone and then dried under vacuum.

We suspended the beads in MeCN and then piped the solution of beads into the microfluidic chip while the "column valve" was actuated. The square profile fluidic channel necessary for the construction of "column valves" ensures that the thin membrane can not close fully. While liquid are able to flow through small openings at the two edges, solids objects remain trapped by the "column valve". A column of porous beads was thus packed inside the reaction chamber. All the phosphoramidite compounds were dissolved into anhydrous MeCN to form 0.1 mol/L solutions. The CSO was dissolved in anhydrous MeCN (0.1 g/mL) and filtered through a 0.45 μm filter.

All the reagents were flushed through the microfluidic channels during setup to remove unwanted air bubbles. Each synthesis cycle contained 3 reaction steps and 3 washing steps. The step-sequence was: deblocking (2 min) → washing (1 min) → coupling (2 min) → washing (1 min) → oxidizing (2 min) → washing (1 min) (Scheme

1). During the coupling step, two coupling reagents (phosphoramidite and activator) were sent through the reaction column alternately (Figure S1): one reagent flowed continuously for 1.8 s then the other one flowed for 1.8 s at a flow rate of 500 nL/min. For the labeled oligonucleotides, we replaced one of the phosphoramidite solutions with Cy3-phosphoramidite and increased the coupling time to 5 min. Final "DMT-off" ("MMT-off" for Cy3-phosphoramidite) was done by flowing deblocking reagent into the column for 2 min. After MeCN wash, we released the "column valve" and flushed the beads out of the chip and into a micro-centrifuge tube. The MeCN was evaporated using centri-vap. We added 0.3 mL concentrated ammonium hydroxide into the tube and then incubated the tube at room temperature for 1 hour. The beads were spun down to the bottom of the tube by centrifuge and then the liquid phase was transferred into a new tube and kept at room temperature for 3 hours for removal of the side-protection groups on the oligonucleotides. Finally the tube was lyophilized to yield solid-form oligonucleotides (with salts).

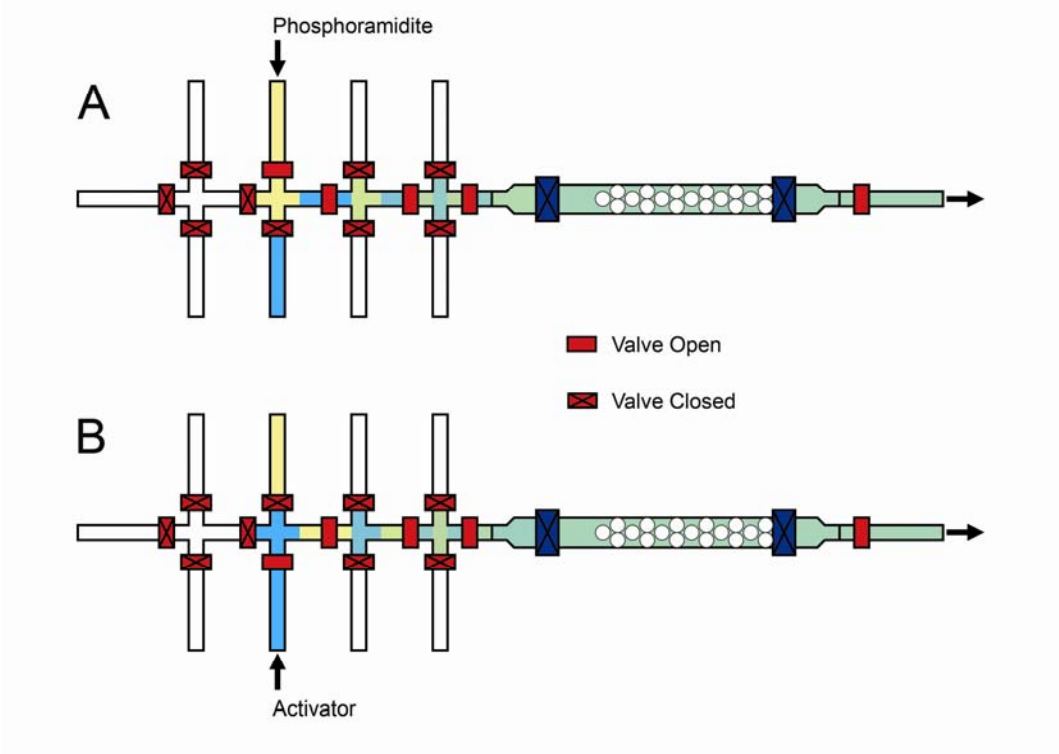

Figure S1. Schematic diagram of the coupling step. (A) Phosphoramidite is flowing through the column. (B) Activator is flowing through the column.

6. Electrophoresis

The synthesized oligonucleotides were re-suspended into pH 7.5 TE buffer and mixed with TBE-Urea sample loading buffer (Invitrogen). The samples (typical volume is 20 μL) were loaded onto a TBE-Urea gel. 1X TBE running buffer (Invitrogen) was used. The temperature was set to ca. 60 ºC and the voltage was set to 175 V. Usually running time of 45 min to 1 hour was enough to achieve single-base resolution of the gel bands. For un-labeled samples, we used SYBR gold dye to stain the gels for 30 min. A Typhoon 9410 (GE Health) scanner was used to scan the gel images. The final images were processed using Matlab (Mathworks).

7. Mass Spectrometry

The synthesized oligonucleotides were sent to Novatia, LLC (Monmouth Junction, NJ) for HPLC/MS characterization. The LC column was a 2 × 50 mm Clarity C18 3 μm from Phenomenex and the experimental temperature was 40 °C. The gradient was 5-25% B in 20 min at 200 μL/min. A = 0.075% hexafluoroisopropanol (HFIPA) / 0.0375% DIEA in water, B = 90% methanol with 0.075% HFIPA / 0.0375% DIEA. The MS data were acquired on an LTQ mass spectrometer from Thermo. The samples were analyzed by electrospray ionization (ESI) mass spectrometry. The detected signal was deconvoluted to generate the final mass spectra.

8. Melting curve measurement

We used a commercial microfluidic digital isolation and detection (DID) chip (Fluidigm, South San Francisco, CA) to carry out melting curve measurements. 1 pmol of standard DNA sample or the DNA sample synthesized from the microfluidic chips (same sequence, with 5'-Cy3 labeled, 5'-Cy3-CCG ACC TGG ATA CTG GCA TT-3') and 1 pmol of FAM-labeled complementary strand (5'-AAT GCC AGT ATC CAG GTC GGT TT-FAM-3') or 1 pmol of FAM-labeled single nucleotide mismatched strand (5'-AAT GCC AGT AAC CAG GTC GGT TT-FAM-3') were mixed with Taq PCR buffer (doped with VOX dye for internal reference) to form 10 μL solutions. The complementary and single nucleotide mismatched strands had 3 extra Thymidine bases at the 3'-end because the fluorescent quantum efficiency would decrease drastically if the FAM was directly coupled to Guanosine. Negative control experiments (solutions containing only FAM-labeled samples or only Cy3-labeled samples, or none of the labeled samples) were also

carried out on the same chip simultaneously. We heated the samples to 95 ºC for 5 min and then placed the chips on a lab bench to cool to room temperature in order to hybridize the DNA strands in the solutions.

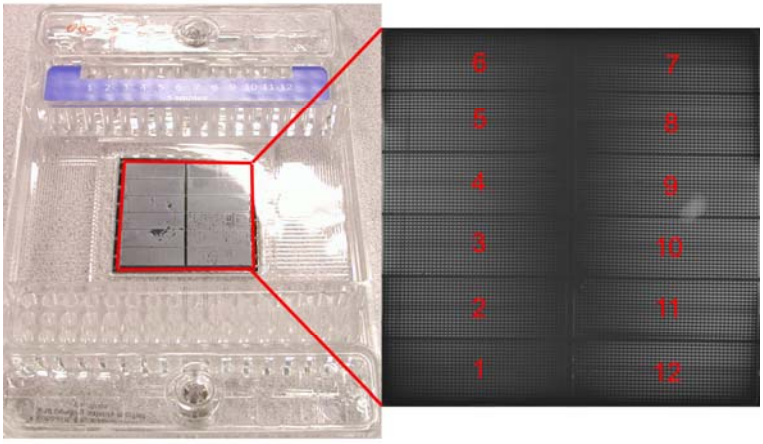

Figure S2. The DID chip and a sample fluorescent image of the chip. Each bright square section in the fluorescent image indicates an isolated reaction well.

We loaded the samples into the chip by following the manufacture's instructions. Each sample was delivered into 1200 isolated identical reaction cells (Figure S2). The chip was placed on a computer-controlled thermo-cycler and was excited by a band-filtered mercury lamp. The fluorescent signal was captured by CCD camera and both FAM and VOX channels were recorded. We cooled down the chip to 15°C and then slowly increased the temperature from 15 ºC to 75 ºC. We captured the images while the chip is heated. The VOX image at each temperature was used to correct for in-homogeneity of the excitation intensity over the chip area (~ 4 cm × 4 cm). The fluorescent signal for each sample integrated over ~250 individual cells was used for the data processing. Each intensity point was calibrated with VOX channel signal,

background-corrected with the negative control experiments, and then normalized as Figure 2 in the paper.

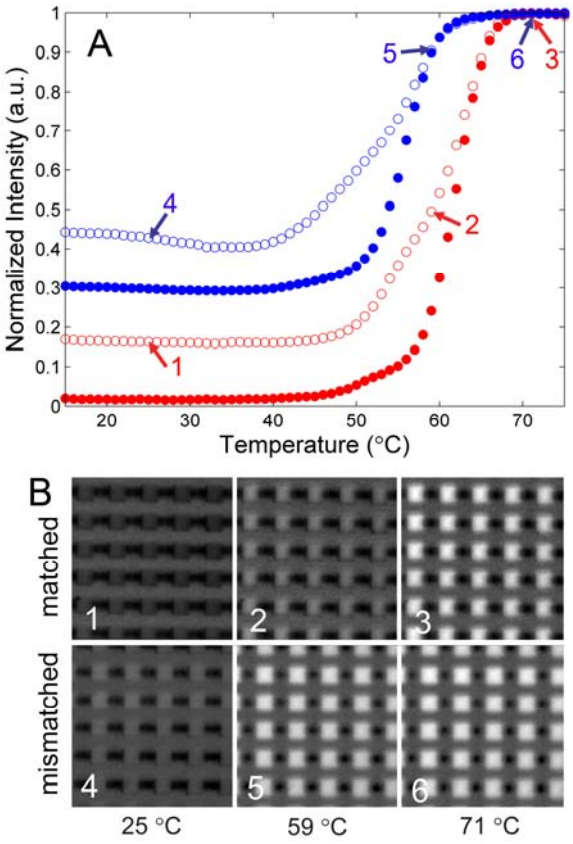

Figure S3. Fluorescent images of unpurified synthesized ss-DNA mixed with complementary and mismatched strands at different temperature on a PDMS chip with multiple reaction wells. Each bright square section in the image is an individual sample well.

II. Additional Results

1. TBE-Urea Gel electrophoresis.

(1) 5'-Cy3-TTT TTT TTT TTT TTT TTT TT-3' (Poly-dT 20-mer, Cy3 labeled)

We also synthesized poly(dT) DNA oligonucleotides (20 mer, with Cy3 labeled at the 5'-end) and compared it to the standard sample purchased from IDT. The standard sample is a mixture of identical amounts of the HPLC purified poly(dT) 10mer, 15mer and 20mer. The gel image (Figure S4) indicates that the major product of our synthesis is the poly(dT) 20mer.

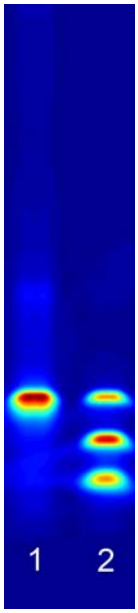

Figure S4. Lane 2 is the mixture of the HPLC purified Cy3-labelled poly-dT 10-mer, 15-mer and 20-mer (all ordered from IDT). Lane 1 is the 5'-Cy3-labeled poly-dT 20-mer sample we synthesized from the PFPE microfluidic chip, without purification.

(2) 5'-CCG ACC TGG ATA CTG GCA TT-3' (DNA 20-mer)

We also synthesized the DNA 20-mer without fluorescent labels. We use both gel electrophoresis and LC/MS to test our product. Figure S5 is the gel image. Figure S6 and S7 are LC/MS data. The gel was stained with SYBR gold. Lane 2 contains 1 pmol of DNA molecules, while lane 1 contains 10% of the product from a single reaction on the microfluidic chip. By comparing the fluorescent intensity of the bands, we can estimate that the total amount of the DNA oligonucleotides produced by each run of the reaction is ca. 60 pmol. The reaction of the labeled DNA oligonucleotides has a similar yield.

Figure S5. Lane 2 is the HPLC purified unlabeled DNA 20-mer ordered from IDT. Lane 1 is our synthesized unlabeled DNA 20-mer without purification.

2. HPLC/MS result.

Chromatogram Summary

Retention Time (min)	Base Peak Mass (Da)	Intensity	LC/MS Area Percent
14.13	5475.4	1.61E+004	7.57
14.53	5788.2	1.16E+005	24.64
14.74	5803.3	1.92E+005	25.55
14.96	6092.5	8.94E+005	42.24

From the deconvoluted mass spectrums of product from various retention time of HPLC, one can conclude that the major impurities are DNA sequences with deletion of single nucleotides. No obvious results were observed regarding material leached from fluoropolymers. However, due to the inert nature of the fluoropolymer as well as complete curing of the device, leached material should have minimal or no effect on solid-phase synthesis of DNA.

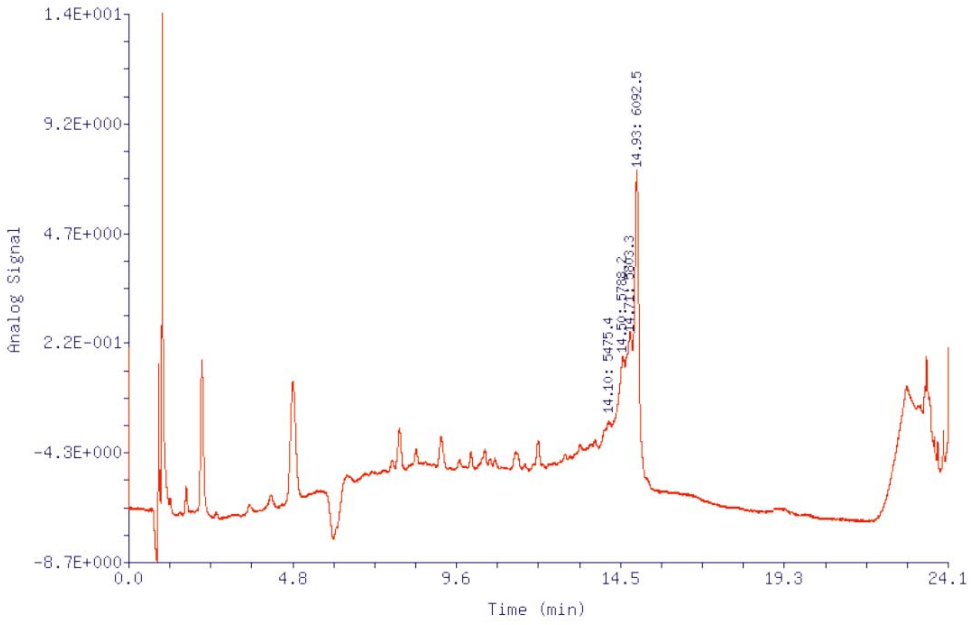

Figure S6. LC/UV chromatogram of the synthesized DNA 20-mer at 260 nm.

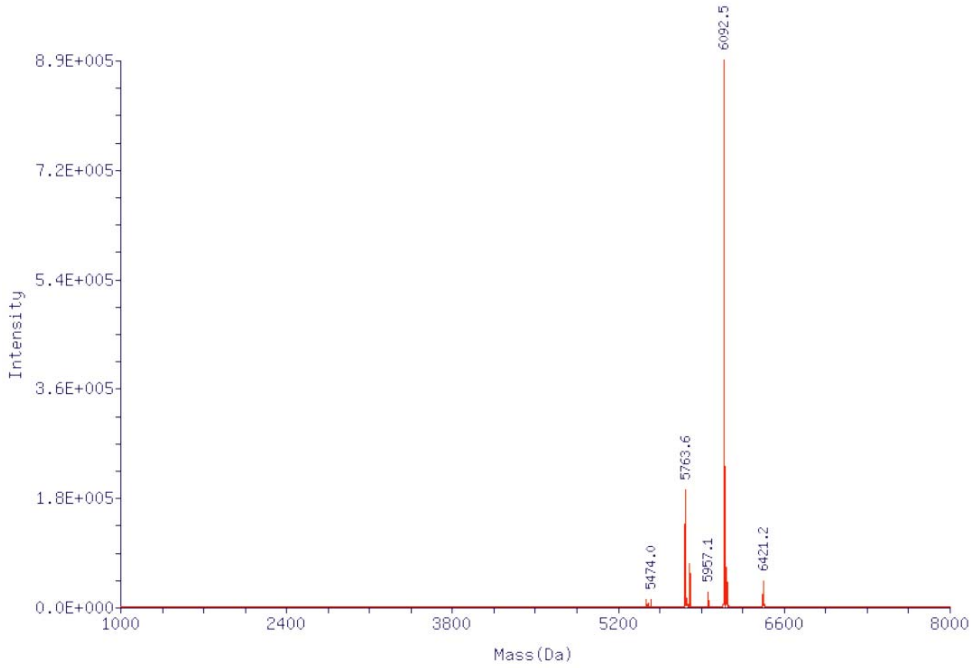

Figure S7. The deconvoluted mass spectrum of the synthetic DNA 20-mer at the retention time of 14.96 min. The calculated molecular weight of the DNA 20-mer is 6093 and the detected molecular weight from MS is 6092.5. The smaller peak with molecular weight of 5763.6 comes from a single deletion of nucleotide G in the expected sequence.

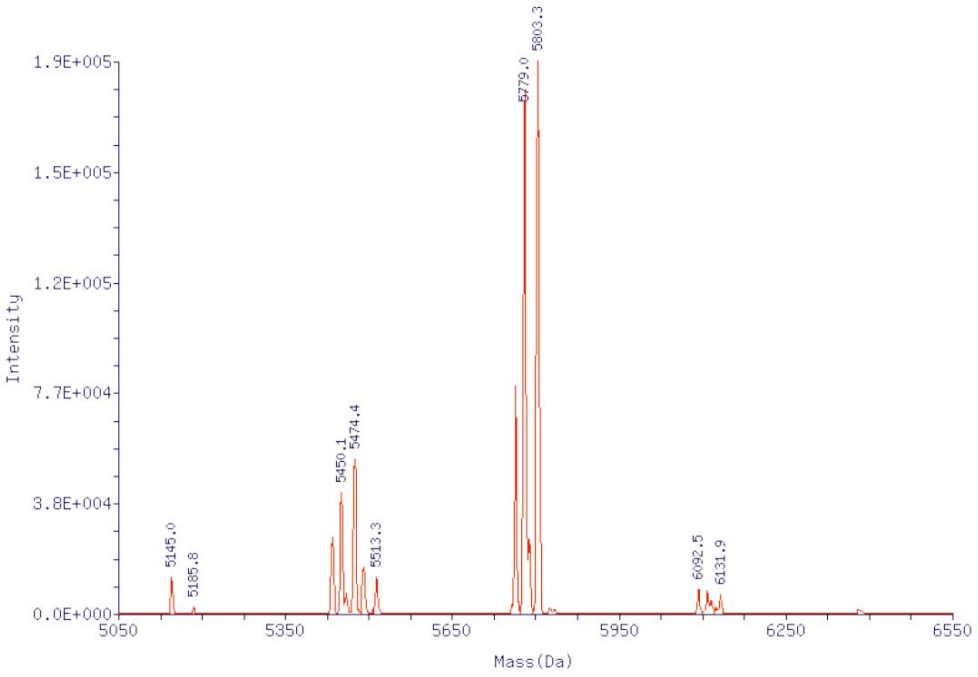

Figure S8. The deconvoluted mass spectrum of the synthetic DNA 20-mer at the retention time of 14.74 min. The two large peaks with molecular weight of 5779.0 and 5803.3 correspond to single deletions of nucleotide A or nucleotide C from the expected sequence, respectively.

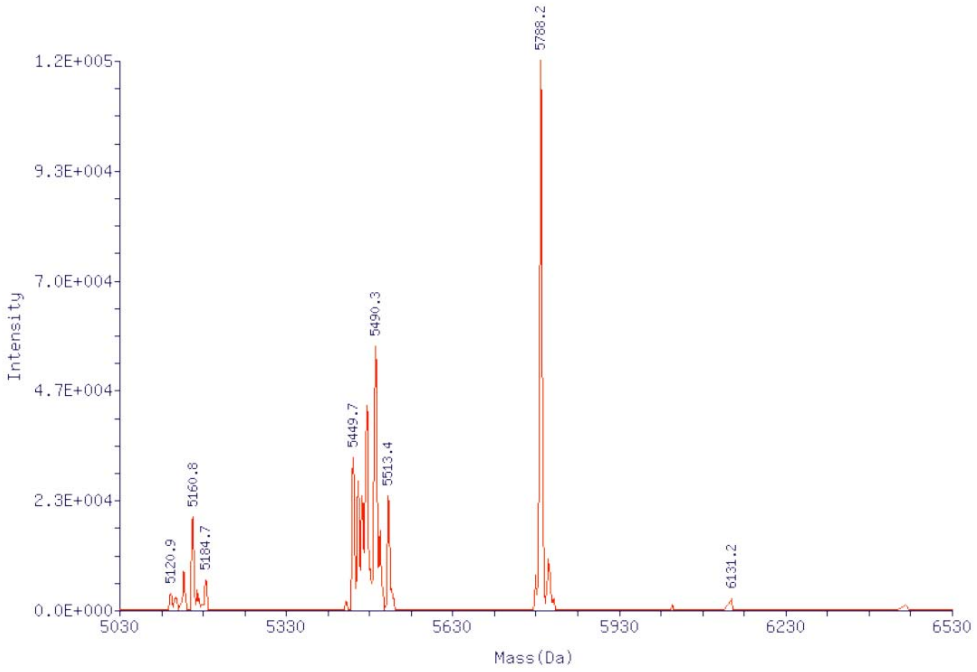

Figure S9. The deconvoluted mass spectrum of the synthetic DNA 20-mer at the retention time of 14.53 min. The large peak with molecular weight of 5788.2 corresponds to a single deletion of nucleotide T in the expected sequence.