3D Printed Fluidic Hardware for DNA Assembly

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Abstract

DNA assembly is a foundational technology for synthetic biology. Microfluidics present an attractive solution for miniaturizing assembly reagent volumes, enabling multiplexed reactions, and interfacing with laboratory automation. However, microfluidics fabrication and operation can be expensive and require expertise, limiting access to an enabling technology. With advances in commodity digital fabrication tools, it is now possible to directly print not only fluidic devices, but supporting hardware as well. 3D printed micro- and millifluidics are easy to make, are low cost, and can be rapidly produced. Here, we demonstrate Golden Gate DNA assembly in 3D printed fluidics with reaction volumes as small as 500 nL, channel widths as fine as 300 micrometers, and device cost ranging from $0.61 to $4.50 per unit. An open-source 3D-printed syringe pump with an accompanying programmable software interface was designed and fabricated to operate the devices. Quick turnaround and inexpensive material costs allowed for rapid exploration of device parameters and offers a potentially disruptive manufacturing paradigm for synthetic biology hardware design and fabrication.

Introduction

Synthetic Biology is a rapidly advancing field that enables novel biotechnology applications, next-generation therapeutics, and new methods of scientific inquiry. However, participation in synthetic biology is largely limited to academic, government and commercial entities. Open, distributed biological innovation in garages, start-up companies and artist studios—similar to today's ecosystem for software innovation—is nascent. Existing barriers to biological innovation have been attributed to several factors, including the high cost of equipment and reagents, intellectual property barriers[1], and difficulty and cost of obtaining genetic consumables (synthesized DNA, plasmids) as an independent person or company[2].

Open biology tools can help overcome these barriers. Open biology tools are typically much lower cost than existing tools, and their design and operation can be co-developed and improved through a distributed, iterative approach. Several open biology tools have already been designed and shared, including thermocyclers (OpenPCR[3]), centrifugation machines(DremelFuge[4]), optics equipment[5], a syringe pump[6], a colorimeter[7], a DNA oligonucleotide synthesizer[8], and a turbidostat[9].

Digital fabrication technologies such as 3D printers, laser cutters, and CNC mills have enabled the design, fabrication, and distribution of these tools. Digital fabrication has become widespread due to the proliferation of low-cost, desktop 3D printers and digital fabrication labs by initiatives such as the FabLab Foundation[10]. Access to digital fabrication tools and open electronics, such as Arduino and Raspberry Pi, enables access to an online and growing set of biology tools that can be downloaded, fabricated and then used (**Figure 1**).

In this work, we developed open fluidic hardware for assembly of DNA-based genetic circuits. Solid-phase DNA synthesis has declined in price, enabling researchers to routinely design and order synthetic DNA up to several kilobases in length. However, assembly of these sequences into larger constructs remains an essential technique. Indeed, DNA assembly is necessary to generate complex single-gene and multi-gene constructs[11–13], to create functionally-diverse part combinations (e.g., gene clusters with libraries of promoters[14]), to "shuffle" homologous proteins at specific recombination points (e.g., shuffling of three trypsinogen homologues[15]), and to explore higher-order effects of genetic architectures (e.g., the position and orientation of transcription units). Recent advances in one-pot DNA assembly methods, such as Golden Gate assembly[16], have made the generation of genetic constructs simpler. But barriers to entry—such as expensive reagents and fluidic handling—continue to make DNA assembly inaccessible for many outside of traditional industry or academic institutions.

Researchers have developed new hardware, electronics and software systems for miniaturizing reagents and fluidic handling for DNA assembly. In particular, DNA assembly has been demonstrated in microfluidic devices, reducing reaction volumes as low as 500 nL[17,18,19]. These devices were fabricated using polydimethylsiloxane (PDMS) replica molding, the most commonly used approach for microfluidic fabrication. However, design and fabrication are still time-consuming and expensive, with a recent study estimating that the production of a new single layer PDMS microfluidic costs $215 and takes 1 day[20]. Microfluidics also require interfacing hardware, such as gravity pumps, compressor lines, and syringe pumps, to route liquids through the devices. The cost and complexity of fabricating and using PDMS microfluidic devices has slowed adoption even though the technology shows tremendous potential for the miniaturization and fine control of liquids. Most

microfluidics publications are still in engineering journals (85% in 2010), rather than biological or biomedical journals[21]. Thus, complexity and cost of fabricating PDMS microfluidics limits the application of this technology to create open tools for DNA assembly.

3D printing of microfluidics provides a potential one-step manufacturing approach for fabricating microfluidic devices to run DNA assembly reactions. Micro- and millifluidics have been printed using fused-deposition modeling (FDM) of thermoplastics[22] and stereolithography (SLA) and inkjet printing of photo-curable polymers[23,24,25,26,27,28,20,29]. Devices have been built for less than $1 per device in only a few hours[22,27]. Numerous desktop FDM and SLA printers are marketed between $300 to $5000[30,31,32]. On-demand 3D printing services, such as Shapeways, can print objects in a range of plastic polymers at a variable cost between $0.28-2.39 per cubic centimeter[33]. Internal channels diameters in 3D printed microfluidics have been reported as low as 100 microns[26]. However, DNA assembly has not been demonstrated in 3D printed fluidics and the compatibility of 3D printed materials with nucleic acids has not been studied.

In this work, we demonstrate the design and use of open, 3D printed fluidic hardware for DNA assembly (**Figure 2**). 3D printed milli- and microfluidics were iteratively designed, fabricated and tested. The fluidics were printed using two methods: the Form1+ (Form Labs, Somerville, Massachusetts), a desktop stereolithography 3D printer, and Shapeways (Shapeways, New York, New York), an on-demand 3D printing service. The milli and microfluidic devices had minimum channel widths of 300 microns and reactor volumes as low as 900 nL. The micro and millifluidics were designed to mix linear double stranded DNA with enzymes to assemble plasmids via Golden Gate biochemistry. Assembly occurred at room temperature, eliminating the need for an incubator. These assembled plasmids were transformed into *E. coli* and the number of transformants was found to be similar to a tube control. The 3D printed fluidics were operated by a bespoke syringe pump (**Figure 2**). The mechanical components of the pump (**Supplementary Figure 6**) were 3D printed using a MakerBot Replicator 2 (MakerBot Industries, Brooklyn, New York) and the pump was controlled by a custom control board (**Supplementary Figure 8**) fabricated using a Roland Modela MDX-20 CNC mill (Roland DG Corporation, Hamamatsu, Japan) running Arduino firmware. A user interface (**Supplementary Figure 9**) enabled the user to control the pump via USB (**Supplementary Figure 5**). Rapid & collaborative design iterations were enabled by

the use of Desktop 3D printers and on-demand printing services. Multiple designs of each fluidic device were required to arrive at the final forms (**Supplementary Figure 4**). The final designs of the 3D printed micro and millifluidic mixers, the syringe pump, and the control software have now been made open via this publication and our hope is that they are downloaded, printed, used, and improved upon by other researchers.

Results

3D printed micro and millifluidic device design and characterization

Three fluidic devices were designed and fabricated to conduct DNA assemblies: a Co-Laminar Mixer printed on the Form 1+, (2) a 3D Micromixer printed on the Form 1+, and a Co-Laminar Mixer printed using SW-FUD (**Figure 3**). Each printing method has advantages. The Form 1+ affords rapid and inexpensive design iterations and near optical clarity using the "Clear" Photoactive Resin. The build volume of the Form1+ is 125 x 125 x 165 mm (X x Y x Z) and the manufacturer claims a minimum lateral laser beam size of 10 microns and feature size of 300 microns[31]. The PreForm software package for the Form1+ offers three z-resolution steps for printing: 25, 50 and 100 microns. We found no measurable difference in resolution between 25 and 50 microns; a 50-micron step size was used for all prints. For printing internal channels, no internal supports were required.

Frosted Ultra Detail is the highest resolution material offered by Shapeways. It is a UV-cured acrylic polymer. The minimum embossed or engraved feature for SW-FUD is 100 microns. The printer accuracy is +/- 25 – 50 microns for every 2540 microns[34]. Frosted Ultra Detail is printed with wax support for external and internal features. This wax support was still present in internal channels printed using SW-FUD and was cleared using ultrasonic cleaning with hot water.

We designed and printed resolution test pieces on the F1+ and using SW-FUD to characterize the fabrication accuracy for both methods and determine the minimum internal channel sizes (**Supplementary Figure 2**). The resolution test piece was designed with 10 circular channels that ranged in diameter from 1.9 to 0.1 mm and 12 square channels that ranged in width from 1.15 to 0.05 mm. All channels were designed to be the length of the test piece, 40 mm. For the Form1+, the smallest diameter circular channel that cleared was 0.9 mm and the smallest square channel that cleared was 0.65 mm (in CAD). For SW-FUD, the smallest cleared circular channel was 0.3 mm and the smallest cleared square channel was 0.25 mm (**Supplementary Figure 2**). These channel dimensions are similar to the minimum channel dimensions reported in previous studies that printed internal channels using SLA and inkjet printing[26,27]. The actual channel dimensions were measured on the exterior of the resolution test piece using optical microscopy (Supereyes USB Digital

Microscope, Shenzhen D&F, Shenzhen, China). For the Form 1+, the measured channel dimension was on average 20 microns greater than the expected dimension (range: 110 to -40 microns). For the SW-FUD, the measured channel dimensions were on average 40 microns smaller then the expected dimension (range: 30 to -90 microns) (**Supplementary Table 1**).

In order to measure the geometry of the internal channels, four sample cross sections of each device were cut and measured (**Supplementary Figure 3**). The Form 1+ devices (**Figure 2 A-B**) featured internal channels that were designed to be 1.5mm in circular diameter and measured to be 1.30 (stdev: 0.09) mm (**Supplementary Table 2**). These devices took 3-4 hours to print and the cost per device was approximately $1. The SW-FUD Co-Laminar device (**Figure 2 C**) was designed to have a 300-micron square channel and a reactor volume of 910 nL. The average side length of the cross sections (**Supplementary Figure I-L**) was 220 microns (stnd. dev. of 30) (**Supplementary Table 2**). Given this average side length, the average reactor volume of the printed SW-FUD devices was 490 nLs (stnd. dev. of 70). The devices cost $5.71 each on Shapeways with a delivery time ranging from 2-10 days.

Devices were designed to mix inputs via co-laminar diffusion. The syringe pump pulled the reagents from the two inlet chambers where they merged at the T-junction and then continued to the outlet of the device. Mixing in the Form1+ and SW-FUD Co-Laminar devices occurred as the two inputs were pulled through the long, primary channel. The design of the Form1+ 3D Micromixer was inspired by previous 3D passive micromixers that have been designed and fabricated using multiple layers of 2D substrates[35]. Mixing was visually confirmed in the Form1+ printed devices by pulling red and blue dyed inputs through the device at a flow rate of 5 µL/s and observing a mixed solution at the outlet.

Iterative design and printing were used to improve device-to-world interfacing and debug practical problems, such as leaks and trapped bubbles. 13 versions of the Form 1+ 3D Micromixer, 5 versions of the Form 1+ Co-Laminar Mixer, and 3 versions of the SW-FUD Co-Laminar Mixer were design, fabricated, and tested (**Supplementary Figure 4**). **Figure 4** shows 5 versions of the Form1+ 3D Micromixer.

Numerous design iterations enabled several innovations that improved usability. In each device, cylindrical chambers were designed to allow the user to pipette the reagents on top of the inlets. Only one control line was used to pull the inputs through the device. The control line was fixed into the device outlet via a press-fit connection. In the SW-FUD device, inlets and outlets were designed to enable a press-fit with a 23-gauge blunt end needle. This connection made it possible to interface the device with a syringe for quick handheld testing and clearing wax from the channels.

Design and testing of the 3D printed syringe pump

We operated the microfluidic devices using an open-source, 3D printed syringe pump (**Figure 2B** and **Supplementary Figures 5-10**). The syringe pump has three components: the mechanical components (**Supplementary Figures 6-7**), a control circuit board (**Supplementary Figure 8**), and a user interface which operates the pump (**Supplementary Figure 9**). The mechanical components consist of a stepper motor, threaded rod, and 3D printed parts. The 3D printed parts were designed in SolidWorks and printed using the Makerbot Replicator 2X. The printed parts are modular, enabling new parts to be printed that enable the operation of different syringes (**Supplementary Figure 7**). The parts designed for this experiment were printed to operate a 1 mL syringe. The material cost of a set of printed parts is $10.11. The electronics were custom designed in EagleCAD and the circuit board was milled using a Roland Modela MDX-20 desktop CNC mill. Surface mounted components, including an Atmel ATMega328P microcontroller and Allegro A3909 stepper motor driver, were soldered onto the milled circuit board (**Supplementary Figure 8**). Custom Arduino-based firmware was written to take commands from a computer via USB and then control the stepper motor (**Supplementary Figure 5**). A user interface was written in Processing to command the pump (**Supplementary Figure 9**). In the interface, the user specified the volumetric flow rate and desired flow volume and commanded the syringe pump to either pull or push fluid. The total cost of the pump was $56.63 and the full bill of materials is in **Supplementary Table 3**. To characterize the performance of the pump, the pump flow rate was measured at 1 µL/sec and 5 µL/sec. The measured flow rate was 1.2 and 3.36% lower than expected (**Supplementary Figure 10**).

Golden Gate DNA assembly in 3D printed fluidics

For each of the three fluidic designs, we tested the efficacy of Golden Gate DNA assembly to construct a bacterial plasmid from two pieces of linear double stranded DNA: a piece encoding a plasmid backbone with a bacterial origin of replication and ampicillin resistance marker, and a piece encoding a constitutive promoter driving expression of yellow fluorescent protein (YFP).

Each fluidic device was used to mix together two volumes: a solution containing both pieces of DNA in ligase buffer and a solution containing BsaI restriction enzyme and T4 DNA ligase in ligase buffer (**Figure 2**). Using the syringe pump, the two solutions were drawn into each microfluidic device and mixed, and then allowed to incubate within the device for 90 minutes at room temperature. We determined the efficiency of on-device Golden Gate assembly by transforming unpurified assembly reaction into *E. coli*, plating serial dilutions on agar media with antibiotics for plasmid selection, and then counting the number of colonies and calculating the colony forming units per microliter of assembly reaction transformed (CFU/μL, Table 1). For all devices, negative controls without enzyme addition yielded no transformants. Positive controls—in which the components were mixed together by pipette and incubated in a PCR tube—yielded an average of 3.2×10^4 CFU/μL. In addition, for each device we incubated reactions that were pre-mixed by pipette to ascertain whether the 3D printed photopolymer material inhibited the assembly. For the Form 1+ devices there was no significant difference in the assembly efficiency compared to the in-tube positive controls. However, the SW-FUD devices yielded 3.4×10^5 CFU/μL for the pre-mix control, greater than the in-tube positive control.

Lastly, we calculated the efficiency of assembly reactions mixed within the microfluidic devices. For the Form 1+ devices (the co-laminar mixer and 3D mixer), there was no difference in efficiency compared the in-tube positive control. However, there appeared to be an increase in the CFU/μL for the SW-FUD devices to 8.1×10^5 CFU/μL for the on-device mix. The observation that SW-FUD devices result in greater CFU/μL for both the on-device mix and the pre-mix control likely does not stem from increased assembly efficiency, but instead from the smaller reaction chamber in the SW-FUD device and decreased DNA assembly transformation volume. The amount of assembled DNA is likely present in a saturating quantity. Therefore, when the number of transformants is normalized by the volume of the DNA assembly reaction to yield CFU/μL, the higher reaction volumes transformed from the Form 1+ devices reduce the effective CFU/μL compared to the SW-

FUD devices. We tested this transformation-saturation hypothesis for the positive control and indeed observed reduced CFU/μL at higher volumes of reaction transformed.

Discussion

This work accomplished three goals: (1) demonstrate a general process of developing Open Biology Tools using CAD design software and digital fabrication tools, particularly 3D printing; (2) characterize the performance of two types of 3D printing, the Form1+ SLA Desktop printer and Shapeways Frosted Ultra Detail, for creating milli- and micro- fluidics; and (3) validate the efficacy of Form 1+ and 3D printed fluidic devices for performing Golden Gate DNA assembly.

The Development of 3D Printed, Open Biology Tools

Over the course of this project, we demonstrated that the speed, simplicity, and low-expense of 3D printing enabled the iterative development of Open Biology Tools (**Figure 1**). Each fluidic design cost less than $5 to print and fluidics printed on the Form1+ typically required a few hours to print. As a result, many iterations of each fluidic device were designed, fabricated and tested. Some design iterations of the 3D Micromixer, fabricated on the Form 1+, can be seen in **Figure 4** (all design iterations are in **Supplementary Figure 4**). To develop the syringe pump, numerous versions of the mechanical components and PCB board (S5D) were fabricated using 3D printing and CNC milling. The material cost of the 3D printed parts for the pump was $10.11 (of a total cost of $56.33, **Supplementary Table 3**) and each part was printed in less than 3 hours.

Rapid prototyping and iterative development of the fluidics hardware was used to improve tool usability and performance. The fluidic devices were designed to pull reagents through the outlet tube (**Figure 4 C-E**). Originally reagents were pushed through both inlets (**Figure 4 A-B**), which required carefully aligning the reagents such that they entered the device at the same time. To interface tubing (0.060 in OD, Tygon Microbore, Saint-Gobain, Courbevoie, France) with the fluidic hardware, a press-fit interface (1.5 mm hole with 0.15 mm diameter, 98 degree chamfer) was designed. This interface worked reliably for the Form1+ printed parts; however, leaks occasionally occurred in SW-FUD devices, likely due to the higher pressure required to push fluid through the smaller channel. Other studies have 3D printed standard Luer fittings[27,20,23,28,24] into the devices, which prevent leaks but adds dead volume. A press fit interface was also designed for a 23-gauge dispensing needle inside of the inlets and outlets of the SW-FUD device. This interface was used to clear wax

out of the channels by aspirating the melted wax and hot water through the device. Performance and usability improvements were also made to the syringe pump. All of the mechanical components in the pump (threaded rod, motor, base, mid, top sections, syringes) are fastened using press and screw fits. Each of these fits was designed using multiple rounds of prototyping. The end result, a design without traditional fasteners such as screws, bolts or nails, makes assembly simple (all parts of the pump can be seen in **Supplementary Figure 6**) and enables the tool to be customized using modular elements. Several versions of the syringe pump were created for different syringes sizes by printing modular elements (**Supplementary Figure 7**).

This project is also an example of the development process for Open Biology Tools. The design, fabrication, and implementation of the 3D printed fluidic hardware began in an MIT course in January 2014, and then continued as collaboration between three different MIT labs (Lincoln Laboratory, Synthetic Biology Center, Media Lab). These laboratories each had common 3D printing infrastructure, enabling the exchange of digital design files and local printing & testing. As digital fabrication tools, particularly 3D printers, become mainstream, co-development of open biology tools becomes more possible.

Other than the necessary 3D printing infrastructure, other barriers remain for the co-development of 3D printed Open Biology Tools. First, CAD remains a large barrier for many potential designers. Fluidic devices, in particular, are challenging to design because they require complex 3D fluid networks. The authors of this study had multiple years of experience working with SolidWorks. Many biologists interested in designing fluidics will likely not have similar experience. To overcome this barrier, microfluidic-specific 3D CAD tools could be useful to make fluidic design more accessible and powerful. A tool that uses a library of microfluidic parts that can be combined via drag-and-drop in a visual UI may be particularly useful. Secondly, open tools that require multiple fabrication machines, such as the syringe pump created for this project, are more challenging to fabricate and require additional skills to build. Creating hardware kits that can be purchased, assembled, and modified may buoy the dissemination of these types of tools. The OpenPCR[3] is a successful implementation of this strategy.

Performance of Form1+ and Shapeways-Frosted Ultra Detail for Printing Microfluidics

The second aim of this work was to characterize the performance of the Form1+ and Shapeways-FUD for printing milli- and micro- fluidics. Overall, device performance was consistent with previously reported devices printed using stereolithography and inkjet printing. Two studies published while working on this study used digital light projection (DLP) stereolithography to print square fluidic channels as small as 200-300 microns wide[20,27], smaller than the minimum square channel dimension, 650 microns, printed using the Form 1+. Instead of DLP, the Form 1+ uses galvanometers to steer laser light into the unpolymerized resin. This suggests that desktop DLP stereolithography machines, such as the MiiCraft[36] (MiiCraft, Taiwan), Titan 1[37] (Kudo3D, Pleasanton, CA), and ProJet 1200[38] (3D Systems, Rock Hill, South Carolina), may be more effective than the Form1+ for printing the smallest internal channel dimensions. However, the Form1+ has a larger build area than these desktop DLP printers and has begun marketing flexible resins, which could be used to print elastomeric fluidic devices. Although Shapeways does not publicly state what machines they use for their various materials, the SW-FUD material is likely printed using the 3D Systems ProJet HD 3000 (3D Systems, Rock Hill, South Carolina) [39], a high-resolution inkjet printer. A previous study reported using this printer to make internal square channels between 100-1000 microns[26], consistent with our results.

For both the SW-FUD and Form1+ devices, the geometry of the internal channel cross sections varied from part to part (**Supplementary Figure 3**). This finding was similar to reports from previous studies[20,27]. One of these studies found that print orientation affects the geometry of the printed channels[20]. Print orientation was not controlled for the Form1+ devices and was unknown for the SW-FUD devices. Therefore, it is possible that print orientation was a contributing factor for part-to-part variability.

Efficacy of Form1+ and SW-FUD fluidic devices for performing Golden Gate DNA Assembly

The assembly products from both the Form 1+ and SW-FUD fluidic devices yielded tens of thousands of transformants. The pre-mix controls and on-device mixes produced comparable or greater numbers of transformants as compared to the in-tube controls. The volume of enzyme used to assemble DNA in the SW-FUD device was less than 1/20 of the volume used in either the Form1+ devices or tube controls. The SW-FUD devices yielded higher colonies per microliter of assembly reaction, an observation that can be explained

due to the presence of saturating amounts of assembled DNA, and lower reaction volumes. These results suggest that the 3D printed devices adequately mixed the enzyme and DNA and that the Golden Gate biochemistry proceeds succesfully in these materials and with these geometries. In this work, two pieces of DNA were assembled; however, Golden Gate biochemistry is extensible to larger assemblies and has been used to generate constructs with many pieces. Thus, these devices or similar ones could allow for assembly of more complex genetic circuits using the same method. Finally, the assembly efficiency of these reactions indicates that as printing technology improves and resolution increases, further scaling reaction volumes would still yield a significant number of transformants.

Although these devices, particularly ones fabricated using SW-FUD, miniaturized reagents and performed DNA assemblies, there are notable limitations. The reagent volumes, although significantly smaller, could still be pipetted by a skilled biologist. Several steps before and after the DNA assembly, including PCR of linear DNA and transformation into *E. coli*, are still completed off-device. However, our results set the stage for the development of more complex devices that could integrate several of these steps and potentially parallelize multiple DNA assemblies in a single device.

Conclusions and Outlook

This project serves as an example of how digital fabrication, particularly 3D printing, could enable an open, collaborative design community for biology tools. In this work, we demonstrated the development of 3D printed fluidic hardware for DNA assembly. Similarly, other fluidic tools could be designed, downloaded and printed for specific biological protocols. As highlighted above, limitations exist with printing fluidics using desktop 3D printers and on-demand printing services, particularly print resolution and part-to-part variability. However, 3D printing technology is improving at a rapid pace due to considerable academic and commercial interest, and these limitations may be overcome as technology develops.

Methods

Designing & 3D printing fluidics

All fluidic devices were designed using SolidWorks. STL files were generated in SolidWorks. For 3D printing on the Form1+, STL files were opened in Form Lab's PreForm software. The software was used to orient the devices and generate supports. All devices printed on the Form1+ were printed using Form Labs Clear Resin with a z-resolution of 0.05 mm. For 3D printing using Shapeways, STLs were uploaded to www.shapeways.com and Frosted Ultra Detail material was selected. For printing, the "print it anyway" option was selected.

Post-processing parts to clear channels

To process parts printed on the Form1+ (**Figure 3 A-B**), devices were removed from the build tray and submerged in 70% isopropyl alcohol for 3-5 minutes. Compressed air was blown into each inlet and outlet for 10 seconds to remove alcohol and any uncured resin. Devices were then submerged in 70% isopropyl alcohol again for 1 minute. A syringe was used to wash each channel with isopropyl alcohol. Then, compressed air was blown into each inlet and outlet for 10 seconds. Finally, support material on each device was removed.

To clear wax support material, SW-FUD devices (**Figure 3 C**) were submerged in hot water (95 C) in a Magnasonic ultrasonic cleaner. The devices were cleaned for several minutes. While in the ultrasonic cleaner, a 1 cc syringe (BD, Franklin Lakes, New Jersey) with a 23 gauge, ½" dispensing needle was used to dispense hot water through the channels. After cleaning, channels were tested by hand using the same syringe and needle.

Characterization of Form1+ and Shapeways Frosted Ultra Detail Resolution Test Piece

A resolution test piece (**Supplementary Figure 2**) was designed in SolidWorks. Resolution test pieces were printed on the Form 1+ using clear resin and 0.05 mm z resolution and using SW-FUD. Parts were post-processed following the protocol above. Resolution test pieces were measured using digital calipers. Water with green food coloring was dispensed into each channel using a 1cc syringe with a 23 gauge, ½" dispensing needle to determine if the square and circular channels in the resolution test piece were clear. A USB optical

microscope (SuperEyes B008, Shenzhen D&F, Shenzhen, China) was used to measure the diameter of the circular channels and the side length of the square channels. To determine the scale of each image, a known length on the image was measured using calipers.

Measuring cross sectional geometry of device channels

To determine the dimensions of the internal channels of the Form 1+ and SW-FUD devices, cross sections were measured using a USB optical microscope (SuperEyes B008, Shenzhen D&F, Shenzhen, China). The cross sections were made by taking printed and processed devices and cutting a cross section using high leverage handheld metal snips. The sections were then sanded into a perpendicular surface using a belt sander. Liquid was dispensed and compressed air was blown through the sectioned channel to remove any debris created from sanding.

Design, fabrication, testing & operation of the syringe pump

The syringe pump was designed in SolidWorks. A MakerBot Replicator 2 (Makerbot Industries, Brooklyn, New York) was used to print parts in polylactic acid (PLA). The pump used a NEMA 17 bipolar stepping motor with 200 steps per revolution (Evil Mad Scientist LLC, Sunnyvale, California), a 3/8" threaded rod with 1/12" in pitch (McMaster-Carr, Elmhurst, Illinois) and a custom electronics board designed in Eagle (Cadsoft Computer GmbH, Pleiskirchen, Germany). To create the circuit board, 4" x 6" FR1 circuit board blanks (Inventables, Chicago, Illionis) were milled using a Roland Modela MDX-20 CNC mill (Roland DG Corporation, Hamamatsu, Japan). The electronic components were then soldered into place. The circuit board was powered by a USB FTDI cable, connected to a MacBook Pro laptop. The Atmel Atmega328P microcontroller was bootloaded with Arduino firmware using a AVR in-system programmer. A full bill of materials of the pump is in **Supplementary Table 3**. The circuit board was then programmed and powered via USB FTDI. A user interface for the pump was written in Processing.

To operate the syringe pump, the user connected and powered (5V) the pump to a laptop via USB. The desired flow rates & flow volume were then inputted into the pump. The precise operation of the pump can be seen in **Supplementary Figure 5**.

The performance of the syringe pump was characterized by pumping water at 1 and 5 µL for a specific flow volume and measuring the time and actual flow volume. Time was recorded using a handheld timer; time was measured between when the "push" button on the user interface and the pump stopped moving. Flow volume was measured by weighing the amount of liquid that was pushed out of a tube and onto a piece of parafilm, using a high-accuracy scale.

Preparing fluidics for biological protocol

Devices printed on the Form1+ were additionally cured under a 15 watt fluorescent UV lamp for 24 hrs to allow for additional curing.

30 minutes prior to running the biological experiment, both devices were blocked with bovine serum albumin (BSA). BSA was added by hand into each device using a syringe. After 30 minutes, BSA was removed from the channels by using pressurized air.

World-to-device interfacing

Inlets of all fluidic devices were designed to allow pipetting reagents into inlet wells. Outlets were designed to press-fit with a Tygon Microbore tube (0.060" outer diameter). The press-fit outlet-tube connection was checked for leaks. In the case of a leaky fit, parafilm was used to create an airtight fit. The outlet tube (Tygon Microbore, 0.060" OD, 10-15 cm long) was connected to a 23-gauge, ½" dispensing needle tip & syringe (1 mL BD Luer Lok) and loaded into the syringe pump.

Amplification and purification of DNA parts

The plasmid backbone encoding an ampicillin-resistance cassette and a p15A origin of replication was PCR amplified using primers that comprised (from 5′ to 3′): a BsaI restriction enzyme recognition site, a 4bp assembly scar, and a backbone-annealing region. Forward primer: CGCGGGGGTCTCCAATGCCGTCTTCGCTTCCTCGCTC; reverse primer: GGTGCAGGTCTCGAAGCTGGTCTTCCAGTACAATCTGCTCTGATG.

The YFP cassette encoding the bacterial P_{Tac} promoter, ribosomal binding sequence, yellow fluorescent protein coding sequence, and a transcriptional terminator was amplified using primers that comprised a BsaI restriction enzyme recognition site, a 4bp assembly scar, and an insert-annealing region. Forward primer: CTAGCGGGTCTCAGCTTAACGATCGTTGGCTGTGTTGACAATTAATCATCGGC; reverse primer: TGCCCAGGTCTCTCATTGGACCAAAACGAAAAAAGGCCC.

PCR mixtures were set up that contained 25 µL KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington, MA), 100 pmol of each DNA oligonucleotide primer (Integrated DNA Technologies, Coralville, IA), 23 µL of sterile water, and 0.1 ng of double-stranded DNA template. The reactions were thermocylced according to the following program: (1) 95°C for 3 min, (2) 98°C for 20 seconds, (3) 65°C for 15 seconds, (4) 72°C for 1 minute, (5) repeat steps 2-5 25 times, (6) 72°C for 2 min. PCR amplicons were electrophoresed on a 1% agarose gel , excised with a scalpel, and purified using a Zymoclean Gel DNA Recovery Kit (Zymo Research, Irvine, CA) and eluted in sterile water. The concentration of each purified PCR product was measured using a NanoDrop 1000 Spetrophotometer (NanoDrop, Wilmington, DE).

Golden Gate assembly setup

The Golden Gate assembly reaction was set up into two reaction halves: the first containing DNA in ligase buffer, and the second containing enzymes in ligase buffer. These reaction halves were mixed in subsequent steps. Enzymes and buffer were purchased from New England Biolabs, Ipswich, MA.

DNA solution composition (per reaction):
40 fmol linear plasmid backbone amplicon
40 fmol linear YFP insert
1 µL 10X T4 DNA ligase reaction buffer
Sterile H_2O to 10 µL

Enzyme solution composition (per reaction):
1 µL BsaI
1 µL T4 DNA ligase

1 µL 10X T4 DNA ligase reaction buffer
7.5 µL sterile H_2O

Experimental protocol for running devices printed using the Form 1+

Before beginning experiments, devices were printed, processed, prepared for the biological protocol and interfaced with tubes to the syringe pump (see protocols above). 10 µL of DNA solution and 10 µL of enzyme solution were pipetted into separate inlets. 40 µL of mineral oil was pipetted on top of each solution. The reagents were then drawn through the device using the syringe pump at a rate of 5 µL per second and allowed to incubate at the end of the device near the outlet. After incubation, the reaction product was drawn into the outlet tube with the syringe by hand and immediately pushed into a collection tube and heat inactivated at 80°C. The tube was then removed from the device and the product was pushed into a collection tube. For each device, we also tested pre-mixed reactions. 10 µL of DNA solution and 10 µL of enzyme solution were mixed using a pipette in a 1.5 mL Eppendorf tube. The pre-mix was then pipetted into one inlet. 80 µL of oil was pipetted on top of the mixed reagents. The other inlet was capped using parafilm. Pre-mixed reactions were similarly drawn through the device and into the incubation area and allowed to incubate for 90 minutes. A negative control was completed using the same protocol by substituting the 10 µL of enzyme solution with 10 µL of 1X ligase buffer.

Experimental protocol for running devices printed using Shapeways Frosted Ultra Detail

SW-FUD devices were cleared, prepared for biological protocol and interfaced with tubes to the syringe pump. 5 µL of DNA solution and 5 µL of enzyme solution were pipetted into separate inlets. 10 µL of mineral oil was pipetted on top of both solutions to minimize evaporative losses. Using the syringe pump, the two solutions were drawn through the device at a rate of 1 µL per second until liquid emerged from the outlet tube. To ensure that no reaction product from the outlet tube was collected, the outlet tube was removed & replaced with a new tube after incubation. Then, the reaction product in the device, remaining DNA and enzyme solutions and mineral oil in the inlets were drawn out of the device by hand using a syringe and immediately pushed into a collection tube and heat inactivated at 80°C.

Pre-mix reactions and negative controls were also completed in the SW-FUD devices. For the pre-mix reactions, 5 µL of DNA solution and 5 µL of enzyme solution were mixed by micropipette and then the 10 µL mixture was pipetted into one inlet. The other inlet was covered. 10 µL of mineral oil was pipetted on top. The mixture was drawn into the device at 1 µL per second until it could be seen in the outlet tube. Post-incubation the outlet tube was replaced. The remaining pre-mix reaction and oil was removed from the inlet. 10 µL of de-ionized water was added to the inlet. The reaction product in the device and the DI water in the inlet were then drawn into the outlet, pushed into a collection tube and heat shocked at 80 C. A negative control was completed using the same protocol, except 5 µL of ligase buffer was substituted for the enzyme solution.

Transformation and calculation of assembly efficiency

Heat-inactivated assembly mixtures were transformed into One Shot Mach1 T1 Phage-Resistant Competent Cells (E. coli genotype: F- φ80(*lacZ*)ΔM15 Δ*lacX*74 *hsd*R(rK-mK+) Δ *rec*A1398 *end*A1 *ton*A; Life Technologies, Carlsbad, CA). 0.6-5.0 µL of assembly mixture was added to 50 µL of thawed cells, incubated on ice for 35 minutes, heat shocked at 42°C for 30 seconds, placed back on ice for 2 minutes, and then 450 µL of SOC media was added to the cells. The cells were allowed to recover for 1 hour in a shaking incubator at 37°C. 50 µL of recovery outgrowth was diluted ten-fold into 450 µL SOC three times to obtain 1X, 10X, 100X and 1000X dilutions. 150 µL of each dilution was plated onto an LB agar plate with 100 µg/mL ampicillin and allowed to grow overnight at 37°C.

The following day, the number of colonies that grew on the agar plates corresponding to the 1000X dilution were tallied. Colony forming units per µL of assembly transformation was calculated by taking the number of colonies, dividing by the volume of assembly mixture added, and multiplying by 3300 (to account for the dilution factor and plated volume).

References

1. Carlson, R. H. *Biology is Technology*. 179–239 (Harvard University Press, 2010).

2. Schloendorn, J. in *BioCoder Issue 4* (O'Reilly Media, 2014). at <http://chimera.labs.oreilly.com/books/1234000002036/ch02.html#_inaccessibility>

3. OpenPCR. at <http://openpcr.org/>

4. Garvey, C. DremelFuge - A One-Piece Centrifuge for Rotary Tools by cathalgarvey - Thingiverse. (2009). at <http://www.thingiverse.com/thing:1483>

5. Zhang, C., Anzalone, N. C., Faria, R. P. & Pearce, J. M. Open-source 3D-printable optics equipment. *PLoS One* **8,** e59840 (2013).

6. Wijnen, B., Hunt, E. J., Anzalone, G. C. & Pearce, J. M. Open-source syringe pump library. *PLoS One* **9,** e107216 (2014).

7. Anzalone, G. C., Glover, A. G. & Pearce, J. M. Open-source colorimeter. *Sensors (Basel).* **13,** 5338–46 (2013).

8. Lausted, C. *et al.* POSaM : a fast , flexible , open-source , inkjet oligonucleotide synthesizer and microarrayer. **5,** (2004).

9. Takahashi, C. N., Miller, A. W., Ekness, F., Dunham, M. J. & Klavins, E. A Low Cost, Customizable Turbidostat for Use in Synthetic Circuit Characterization. (2015).

10. Gershenfeld, N. How to Make Almost Anything The Digital Fabrication Revolution. **91,**

11. Cermak, T. *et al.* Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Res.* **39,** e82 (2011).

12. Zhang, F. *et al.* lette r s Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription. **29,** 149–154 (2011).

13. Weber, E., Engler, C., Gruetzner, R., Werner, S. & Marillonnet, S. A modular cloning system for standardized assembly of multigene constructs. *PLoS One* **6,** (2011).

14. Smanski, M. J. *et al.* Functional optimization of gene clusters by combinatorial design and assembly. *Nat. Biotechnol.* **32,** (2014).

15. Engler, C., Gruetzner, R., Kandzia, R. & Marillonnet, S. Golden gate shuffling: A one-pot DNA shuffling method based on type ils restriction enzymes. *PLoS One* **4,** (2009).

16. Engler, C., Kandzia, R. & Marillonnet, S. A one pot, one step, precision cloning method with high throughput capability. *PLoS One* **3,** e3647 (2008).

17. Kong, D. S., Carr, P. a, Chen, L., Zhang, S. & Jacobson, J. M. Parallel gene synthesis in a microfluidic device. *Nucleic Acids Res.* **35,** e61 (2007).

18. Huang, M. C., Ye, H., Kuan, Y. K., Li, M.-H. & Ying, J. Y. Integrated two-step gene synthesis in a microfluidic device. *Lab Chip* **9,** 276–85 (2009).

19. Quan, J. *et al.* Parallel on-chip gene synthesis and application to optimization of protein expression. *Nat. Biotechnol.* **29,** 449–52 (2011).

20. Au, A. K., Lee, W. & Folch, A. Mail-order microfluidics: evaluation of stereolithography for the production of microfluidic devices. *Lab Chip* **14,** 1294–301 (2014).

21. Sackmann, E. K., Fulton, A. L. & Beebe, D. J. The present and future role of microfluidics in biomedical research. *Nature* **507,** 181–9 (2014).

22. Kitson, P. J., Rosnes, M. H., Sans, V., Dragone, V. & Cronin, L. Configurable 3D-Printed millifluidic and microfluidic "lab on a chip" reactionware devices. *Lab Chip* **12,** 3267–71 (2012).

23. Anderson, K. B., Lockwood, S. Y., Martin, R. S. & Spence, D. M. A 3D printed fluidic device that enables integrated features. *Anal. Chem.* **85,** 5622–5626 (2013).

24. Erkal, J. L. *et al.* 3D printed microfluidic devices with integrated versatile and reusable electrodes. *Lab Chip* **14,** 2023–32 (2014).

25. Bhargava, K. C., Thompson, B. & Malmstadt, N. Discrete elements for 3D microfluidics. *Proc. Natl. Acad. Sci.* **111,** 15013–15018 (2014).

26. Lee, K. G. *et al.* 3D printed modules for integrated microfluidic devices. *RSC Adv.* **4,** 32876 (2014).

27. Shallan, A. I., Smejkal, P., Corban, M., Guijt, R. M. & Breadmore, M. C. Cost-E ff ective Three-Dimensional Printing of Visibly Transparent Microchips within Minutes. (2014).

28. Chen, C., Wang, Y., Lockwood, S. Y. & Spence, D. M. 3D-printed fluidic devices enable quantitative evaluation of blood components in modified storage solutions for use in transfusion medicine. *Analyst* **139,** 3219–26 (2014).

29. Krejcova, L. *et al.* 3D printed chip for electrochemical detection of influenza virus labeled with CdS quantum dots. *Biosens. Bioelectron.* **54,** 421–7 (2014).

30. Kickstarter >> The Micro: The First Truly Consumer 3D Printer by M3D LLC. at <https://www.kickstarter.com/projects/m3d/the-micro-the-first-truly-consumer-3d-printer>

31. FormLabs. (2014). at <http://formlabs.com/en/>

32. Spark | Ember 3D Printer. at <http://spark.autodesk.com/ember#about-explorer>

33. Shapeways. (2014). at <http://www.shapeways.com>

34. Frosted Detail Plastic Information. (2014). at <https://www.shapeways.com/materials/frosted-detail-plastic>

35. Liao, Y. *et al.* Rapid prototyping of three-dimensional microfluidic mixers in glass by femtosecond laser direct writing. *Lab Chip* **12,** 746–9 (2012).

36. MiiCraft. at <http://www.miicraft.com/>

37. Kudo3D. at <http://www.kudo3d.com/>

38. ProJet® 1200 | www.3dsystems.com. at <http://www.3dsystems.com/es/projet1200>

39. a visit to Shapeways NY factory! | Nervous System blog. at <http://n-e-r-v-o-u-s.com/blog/?p=3937>

40. Carrière, P. On a three-dimensional implementation of the baker's transformation. *Phys. Fluids* **19,** 118110 (2007).

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Author contributions W.G.P., A.A.K.N., S.J.K., C.W., T.L., J.J.R., O.M., and D.S.K designed the devices. W.G.P., S.J.K., and N.E.O. characterized the two 3D printing fabrication methods. W.G.P, C.W. and T.L. designed, fabricated and tested the syringe pump. W.G.P, A.A.K.N performed the experiments and analyzed the data. W.G.P, A.A.K.N., D.S.K., and C.A.V. designed biological experiments. P.C. and D.S.K. provided initial ideas. W.G.P., A.A.K.N.,

S.J.K., C.W., T.L., J.J.R., O.M., N.E.O, C.A.V., and D.S.K contributed to writing and editing the paper.

Device	N	Mean CFU per uL (10^4)	Sta. Dev. (10^4)
Tube			
Neg	4	0.00	0.00
Pos	7	3.20	1.61
Form1+ co-laminar			
Neg	1	0.00	0.00
pre-mix	6	2.90	1.57
on-chip mix	5	2.60	2.41
Form1+ micromixer			
Neg	1	0.00	0.00
pre-mix	5	3.48	2.83
on-chip mix	7	3.52	3.08
Shapeways co-laminar			
Neg	1	0.00	0.00
pre-mix	5	8.10	5.80
on-chip mix	7	7.80	4.90

Table 1 | Colony forming units of *E.coli* transformed with golden gate products and grown on ampicillin plates for each of the 3D printed fluidics

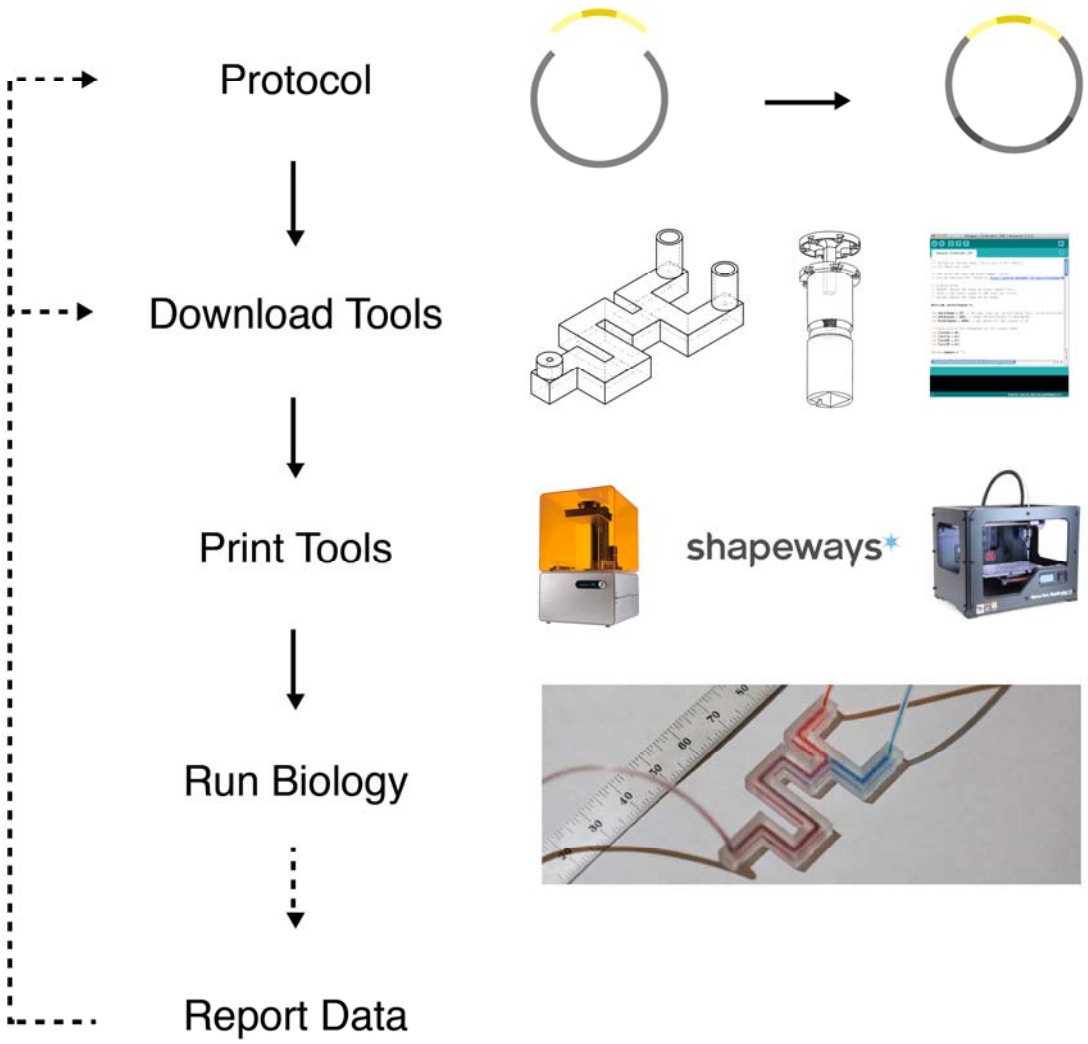

Figure 1 | Designing, Distributing and Fabricating Open Biology Tools. Digital fabrication tools enable on-demand fabrication of biology tools. This capability allows a researcher to fabricate biology tools needed for a protocol of interest by downloading CAD files and software and "printing" the tools using digital fabrication tools, such as desktop 3D printers and on-demand, online printing services. Results from running experiments using these tools can be fed back into the tool design for iterative improvement.

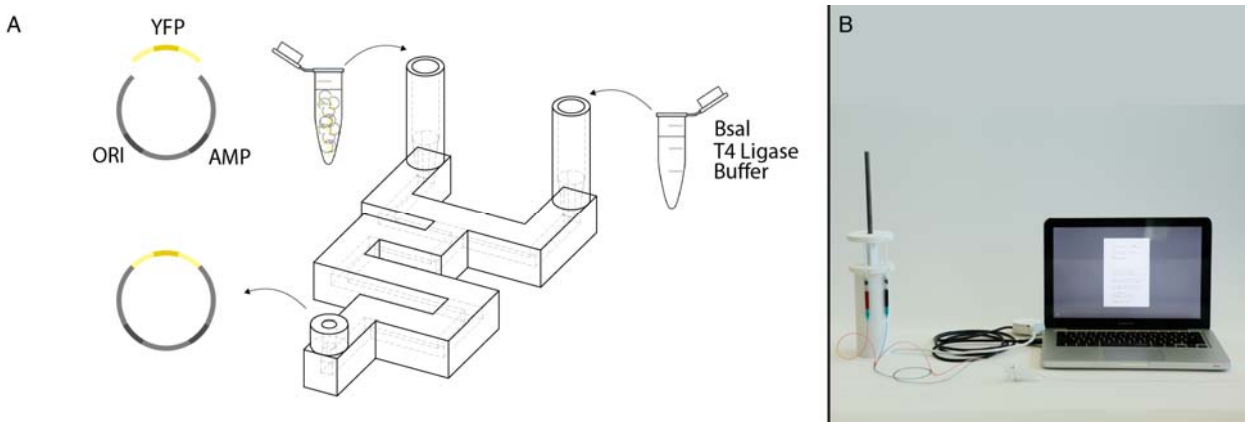

Figure 2 | DNA assembly using 3D printed fluidics hardware. (**A**) Linear DNA was assembled into plasmids using the Golden Gate method inside of fluidics hardware. Two linear DNA segments were used, a constitutively expressed YFP reporter, and a backbone containing an ampicillin selection marker. Each linear segment was PCR amplified prior to assembly. The linear segments were inserted into one inlet of the fluidic device. Golden Gate reagent mix, BsaI, T4 Ligase, and T4 buffer, was inserted into the other. The two inputs were pulled into the device and mixed via co-laminar diffusion. After a 90-minute room-temperature incubation, the product was pulled the device and then transformed into *E. coli*. This experiment was run on all three device designs (**Figure 3**). To control for device mixing efficacy, assemblies were also run in the devices in which the linear DNA segments and Golden Gate enzyme mix were mixed off-device prior to the experiment. Detailed visual protocols for running this experiment in the SW-FUD devices can be seen in **Supplementary Figure 1**. (**B**) A bespoke syringe pump and user interface designed and fabricated for this experiment. The syringe pump was used to pull the two inputs through the device at 1 and 5 uL/sec. The syringe pump is composed of several parts (**Supplementary Figure 6**), including 3D printed mechanical pieces, a custom designed and fabricated circuit board (**Supplementary Figure 8**), and a software user interface (pictured in **B** and **Supplementary Figure 9**). The CAD designs and software for the syringe pump have been made openly available. The total cost of the pump was $56.33 (**Supplementary Table 3**) and the 3D printed components can each be printed in less than 3 hours.

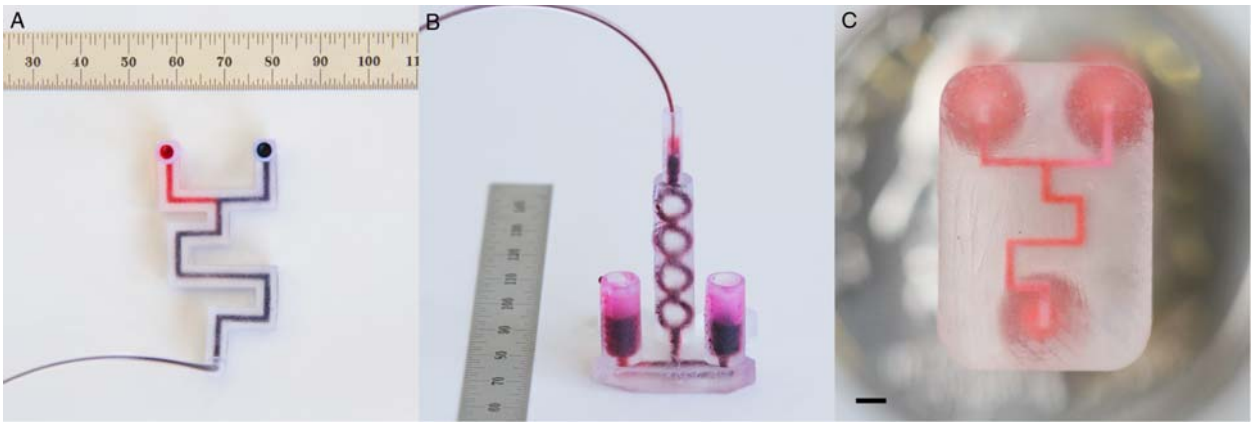

Figure 3 | Fluidics printed using the Form 1+ and Shapeways Frosted Ultra Detail. DNA assembly (**Figure 2A**) efficacy was tested in three fluidic device designs: (**A**) the Form 1+ Co-Laminar Mixer, (**B**) the Form 1+ 3D Micromixer, and (**C**) the SW-FUD Co-Laminar Mixer. The Form 1+ devices were designed with 1.5 mm diameter circular channels and the SW-FUD device was designed with a 300-micron square channel.

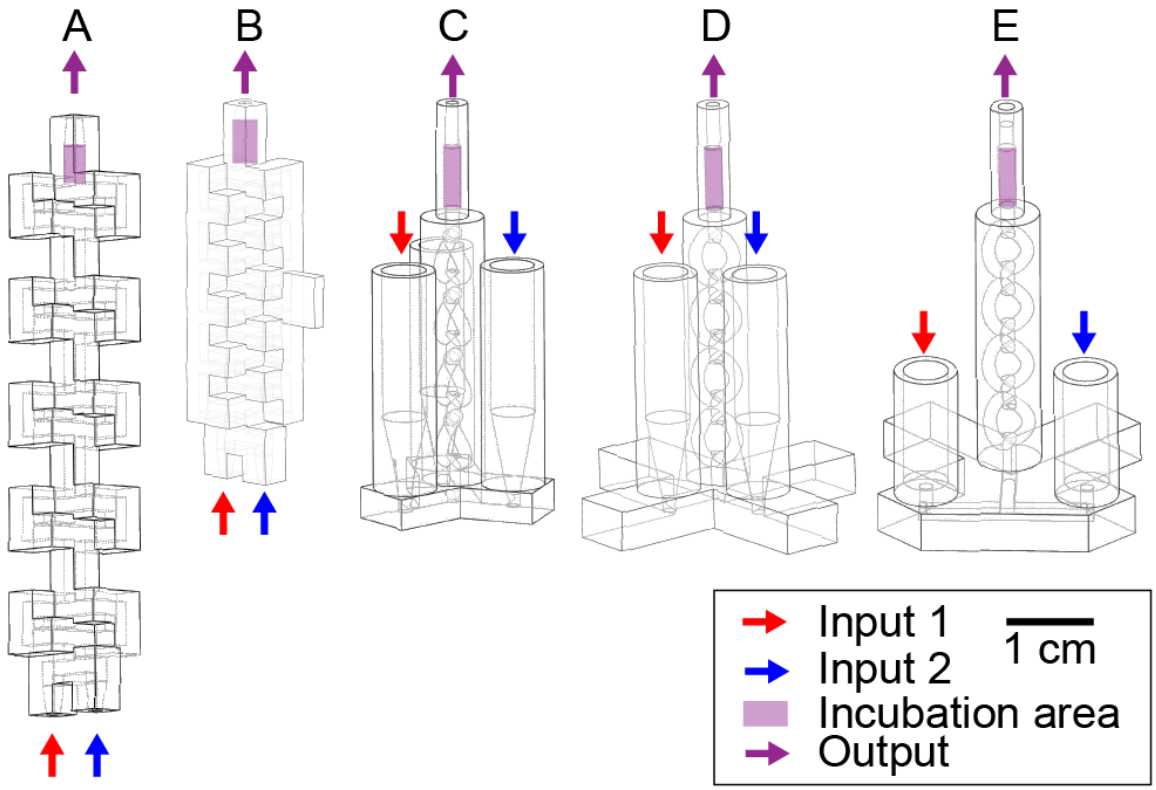

Figure 4 | Design evolution of the 3D Micromixer. Many designs were created and tested for each of the three fluidic devices (**Figure 3**) used in the DNA assembly experiments. 5 (**A-E**) of 13 iterations of the Form 1+ 3D Micromixer (**Figure 3B**) can be seen above. Several design changes can be seen **A-E**: The design of the 3D micromixer was changed from a recti-linear channels with square channels (**A-B**) similar to previous 3d micromixer designs[27,35,40], to a micromixer design using channels with a circular cross-section and smooth curves (**C-E**). A mount held the initial designs (**A-B**); later versions (**C-E**) stood freely on a base. All 13 iterations are in **Supplementary Figure 4**.