Title
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OPTIMIZING THE VIOLACEIN BIOSYNTHETIC PATHWAY USING DROPLET MICROFLUIDICS

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ABSTRACT

We have developed a microfluidic platform that uses aqueous droplets suspended in an oil phase as discrete reaction chambers to carry out molecular biology steps. It integrates and automates all critical procedures of synthetic biology including combinatorial gene assembly, transformation by electroporation, addition of selection medium, culture, assay and sorting on a single device.

KEYWORDS: Cell Culture; DNA Assembly; Droplet Microfluidics; Electroporation; Synthetic Biology

INTRODUCTION

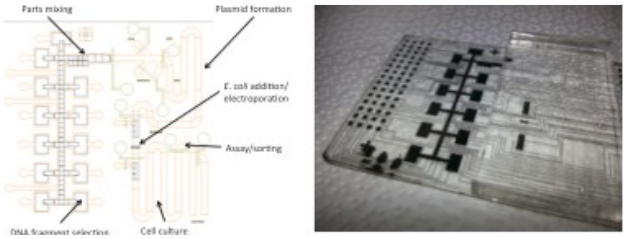
Synthetic biology experiments require optimization of pathways consisting of many genes and other genetic elements and given the large number of alternatives available for each element, optimization of a pathway can require large number of experiments. Currently, these experiments are done using fairly large amounts of costly reagents per experiment making the process very expensive, extremely slow and irreproducible. Our lab has previously developed droplet-based microfluidic systems for automating specific procedures of synthetic biology: gene assembly and electroporation [*1*] or heat-shock and culture [*2*]. However, these operations were performed separately and required additional manual sample preparation to perform the proof-of-principle experiments.

The current system integrates these processes to provide and end-to-end device for optimizing biosynthetic pathways. We applied this platform to screen for the production of violacein, a molecule with applications in textiles, agriculture and found to have antibacterial, antiviral and anticancer behaviors [*3*]. Violacein can be biosynthesized in *E. coli* from L-tryptophan in a process using five enzymes (Figure 1) [*4*].

Figure 1. Schematic of the VioABCDE assembled plasmid (A) and biosynthetic pathway for violacein generation (B).

EXPERIMENTAL

Standard photolithography methods were used to create chrome electrodes and microfluidic channels on a glass substrate [1]. Assembly of the device by sandwiching the microchannels between the electrodes and an indium tin oxide (ITO) film provides a confined



region in the device for creation and manipulation of aqueous droplets by digital microfluidics (Figure 2).

RESULTS AND DISCUSSION

Digital microfluidics was initially used for dispensing and mixing the gene variants, vector backbone and assembly reagents because of the technologies great control of individual droplets and programmability. This component of the device is capable of generating a combinatorial library composed of 25 variants of the 11 kB violacein producing plasmid VioABCDE (Figure 3A,B). Following combinatorial mixing of gene parts the droplets were transferred to a flow-based PDMS channel for all additional procedures: addition of competent cells, electroporation, addition of culture media, culture, fluorescence imaging and sorting (Figure 3 C-F). Thermal regulation across the system was controlled by positioning peltier modules below the device to provide the 4°C, 37°C, 21°C and 50°C temperatures required for cell storage, cell culture and DNA assembly. Following droplet merger the gene parts were assembled at 21°C for Golden-Gate and 50°C for Gibson assembly, respectively. Gene delivery into electrocompetent cells was then accomplished by providing two 200 ms pulses of 1800 V/ cm by the same electrodes employed for digital microfluidics, confirmed by colony PCR and gel electrophoresis (Figure 4). Following addition of culture media and 24 hrs culture, successfully transformed cells exhibited a purple color and strong red autofluorescence, indicative of the production of violacein (Figure 4).

Figure 2. Schematic and image of SynBioChip for automating: combinatorial gene mixing, assembly, electroporation, cell culture and assay.

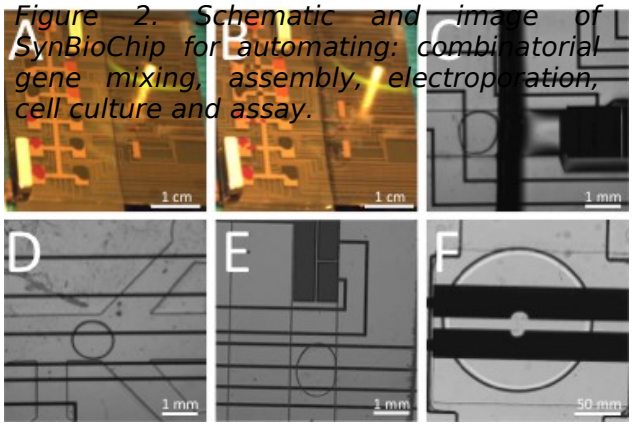


Figure 3. Digital microfluidics is initially employed for the on-demand generation and merging of droplets containing DNA parts and ligation reagents (A,B). Vacuum actuation allows transfer of droplets to a PDMS channel (C) where fluorinated oil is pumped to move droplets through the device (D). Channel electrodes allow merger of droplets containing cells with assembled plasmid (E). Genes are delivered by electroporation (F).

CONCLUSION

The flexibility of digital microfluidics, PDMS valves and peltier modules affords quick optimization of operating protocols. The merger of these technologies provides us with a platform, which can perform a variety of operations with minimal electrical and fluidic inputs. This technology will be of great utility for systematic interpretation of gene delivery methods and high-throughput screening of gene variants with minimal reagent requirements.

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Figure 4. Brightfield and fluorescence images of E. coli cells cultured 24 hrs in the microfluidic device. Droplets containing cells with no added DNA died under kanamycin selection (A,B) whereas cells transformed with the VioABCDE plasmid on the microfluidic device generated a strong autofluorescence (C,D). Following culture on-chip, cells were lysed and DNA parts digested, column purified and PCR amplified. Gel electrophoresis shows definitive bands for each of the DNA fragments (ABC – 5800 BP), (CDE – 3200 BP) and (ABCDE – 7600 BP).

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