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A MICROFLUIDIC PLATFORM FOR COMBINITORIAL GENE ASSEMBLY, TRANSFORMATION, CULTURE AND ASSAY

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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 (Figure 1).

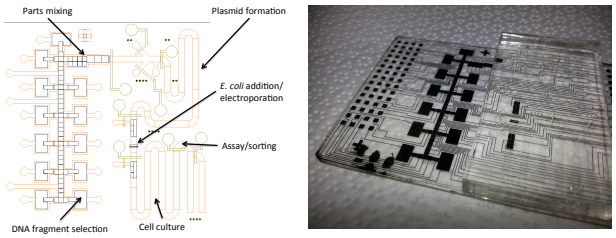

Figure 1. Schematic and image of SynBioChip for automating: combinatorial gene mixing, assembly, electroporation, cell culture and assay.

CCS Concepts

• **Life and medical sciences → Systems Biology** • **Emerging technologies → Electromechanical systems;** *Microelectro-mechanical systems.*

Keywords

Droplet microfluidics; Synthetic biology; Transformation, Cell Culture

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1. 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.

2. Automating Violacein Pathway Screening

The current system integrates these processes to provide an 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 and 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 2) [4]. 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

polydimethylsiloxane channel for all additional procedures: addition of competent cells, electroporation, addition of culture media, culture, fluorescence imaging, and sorting (Figure 3 C-F).

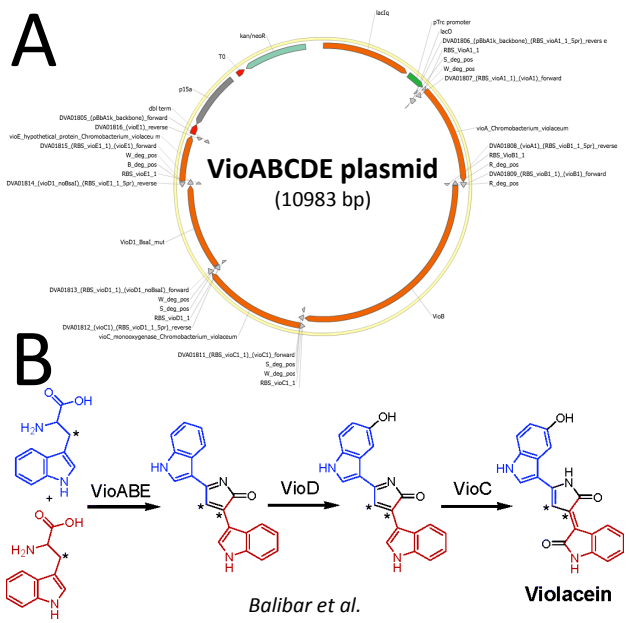

Figure 2. Schematic of the VioABCDE assembled plasmid (A) and biosynthetic pathway for violacein generation (B).

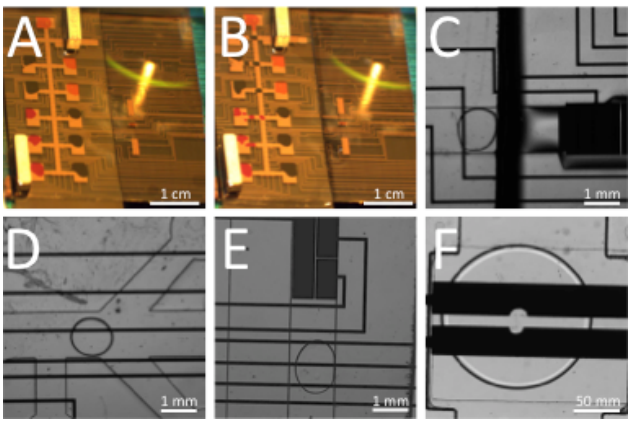

Figure 3. Digital microfluidics is initially employed for the on-demand generation and merging of droplets containing DNA parts and ligation reagents (A,B). Vacuum actuation allows transfer of droplets to a polydimethylsiloxane channel (C) where fluorinated oil is pumped to move droplets through the device (D). Channel electrodes allow merger of droplets containing cells with assembled plasmid (E). Genes are delivered by electroporation with 1800 V/ cm pulses (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. 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). 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.

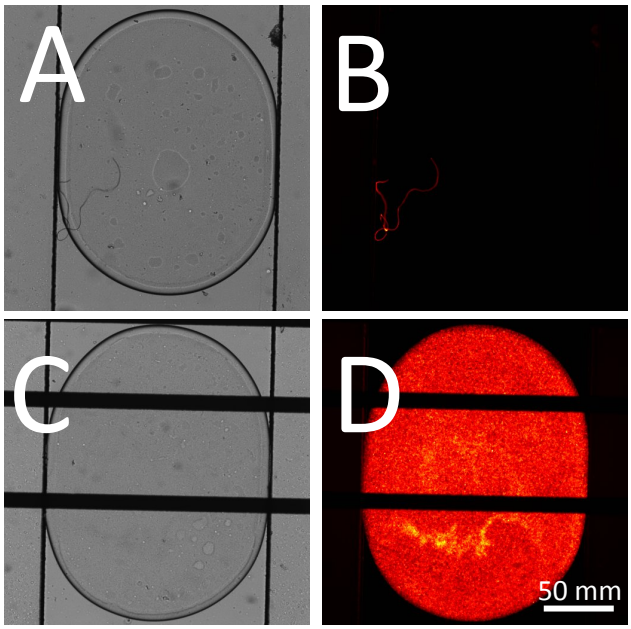

Figure 4. Brightfield and fluorescence images of *E.coli* cells cultured 24 hrs in the microfluidic device. Droplets containing cells with no added DNA died under kanamycin selection (A,B) whereas cells transformed with the VioABCDE plasmid on the microfluidic device generated a strong fluorescence (C,D).

3. References

[1] Shih, S. C.; Goyal, G.; Kim, P. W.; Koutsoubelis, N.; Keasling, J. D.; Adams, P. D.; Hillson, N. J.; Singh, A. K. 2015. A Versatile Microfluidic Device for Automating Synthetic Biology. *ACS Synth. Biol.* 4 (10) (June 2015), 1151-1164.

[2] Gach, P. C.; Shih, S. C.; Sustarich, J.; Keasling, J. D.; Hillson, N. J.; Adams, P. D.; Singh, A. K. 2016 A Droplet Microfluidic Platform for Automating Genetic Engineering. *ACS Synth. Biol.* 5 (5) (Feb 2016), 426-433.

[3] Choi, S. Y.; Yoon, K. H.; Lee, J. I.; Mitchell, R. J. 2015 Violacein: Properties and Production of a Versatile Bacterial Pigment. *Biomed Res Int*, 2015, 465056.

[4] Balibar, C. J.; Walsh, C. T. 2006 In vitro biosynthesis of violacein from L-tryptophan by the enzymes VioA-E from Chromobacterium violaceum. *Biochemistry* 45 (51), (2006), 15444-57.