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A VERSATILE AND ROBUST DROPLET-BASED MICROFLUIDIC AUTOMATION SYSTEM FOR HIGH-THROUGHPUT OPTIMIZATION OF BIOSYNTHETIC PATHWAYS

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ABSTRACT

We present a novel fully-automated droplet-based microfluidic system to enable programmable combinatorial mixing, electroporation for CRISPR-based gene editing, and high-throughput screening on chip. It is highly robust and compatible with conventional liquid handler systems to interface, enabling 100 different reactions at a time with dramatically lower reagents consumption. Utilizing proposed system, we perform accelerated optimization of biosynthetic pathway of indigoidine in *Escherichia coli* (*E. coli*).

KEYWORDS: Droplet Microfluidics, Electroporation, Gene-Editing, Synthetic Biology

INTRODUCTION

In recent years, synthetic biology has drawn significant interest for both scientific research and industrial applications such as biofuel and pharmaceutical production. One good example is indigoidine, bacterial natural dye with antioxidant and antimicrobial activities [1]. Synthetic biology process, however, requires multiple iterations of Design-Build-Test-Learn (DBTL) cycles for optimal production of target biomolecules, which is time-consuming and labor-intensive due to low availability of advanced tools and high-throughput workflows.

Here we propose a versatile and robust droplet-based microfluidic platform that enables high-throughput iterations of DBTL cycles (**Fig. 1a**). The heart of our system is a digital microfluidic (DMF) chip that enables reactions in parallel using nL droplets as reaction vessels as described in our earlier publications [2]. In addition to DMF manipulations for mixing and transporting droplets containing biological parts, proposed platform with 100 discrete chambers is capable of parallel electroporation with different conditions, and additional reservoirs allow recovery incubation and screening on chip.

EXPERIMENTAL

Assembled microfluidic chip is shown in **Figure 1b**, and its 384-well based configuration is compatible with state-of-the-art liquid handling robots and eliminates the complex processes of formation and loading of droplets to the chip. Loaded samples in droplets are kept inside the oil preventing the evaporations (**Fig. 1c**), and DMF manipulation enables active mixing and electroporation on-chip (**Fig. 1d**). In addition, our microfluidic chip is manufacturable with commonly used processes, which is suitable for cost-competitive target molecules such as indigoidine.

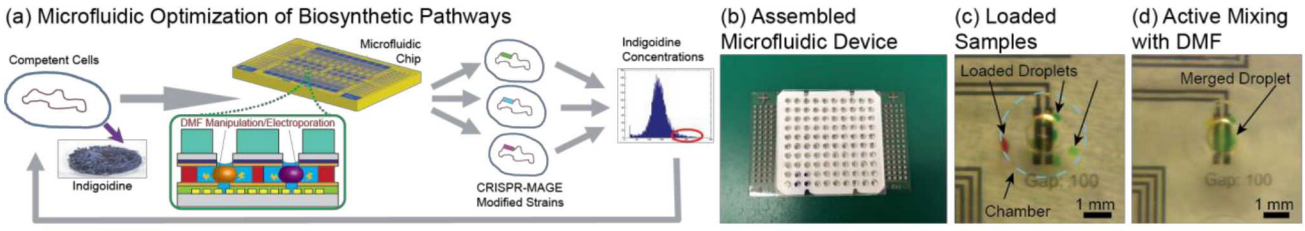

Figure 1: Microfluidic optimization of biosynthetic pathways. (a) concept of the workflows. (b) Fabricated microfluidic devices with 100 reaction chambers in 384 well format. (c) Multiple samples loaded on chip. (d) Demonstration of active mixing of samples in droplets with DMF.

RESULTS AND DISCUSSION

We adapt CRISPR-based MAGE (CRMAGE) recombineering with our platform for efficient gene-editing processes [3], first targeting an enzyme galactokinase (*galK*). *E. coli* colonies on MacConkey plates with red color indicate the wildtypes and white colonies indicate the mutants with *galK* knockout (**Fig. 2a**), confirmed by sequencing the genome. With optimized assay protocols (*e.g.*, concentrations of gRNA and Cas9 inducer, anhydrotetracycline), wildtype killing rate by Cas9 protein achieved over 90% with on-chip transformation.

We then targeted glutamine synthetase (*glnA*) and blue-pigment synthetase (*SFP/bpsA*) enzyme related with indigoidine production (**Fig. 2b**). For the initial round, we designed oligos and gRNA sequence for CRMAGE plasmids targeting T7 promoters [4]. These mutations were screened by antibiotics (kanamycin), and indigoidine production rate with IPTG induction was quantified by measuring absorption spectra at 612nm (**Fig. 2c**). Results clearly indicates the successful production of blue pigments. After quantification, CRMAGE plasmid can be self-eliminated by inducing rhamnose for the next round targeting different loci.

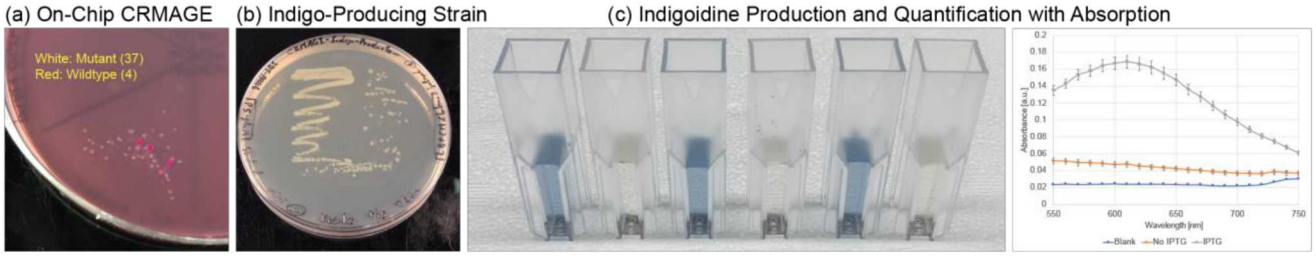

Figure 2: Experimental results of on-chip transformation and indigoidine production. (a) CRMAGE results to knockout GalK. On-chip CRMAGE achieved over 90% of mutation. (b) Modified indigo-producing strain. (c) Successful indigoidine production quantified at OD_{612}. Error bars denote standard deviation of biological triplicate.

CONCLUSION

We have developed a droplet-based microfluidic pathway optimization system, which successfully demonstrated on-chip CRISPR-based gene editing and quantification of indigoidine production rate. With the results shown above, we believe our fully-automated system with capable of 100 reactions at a time would dramatically accelerate the DBTL cycle of biosynthetic pathways for emerging synthetic biology applications.

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