High Throughput Bioengineering Using a Microfluidic Platform

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Project Goals: The JBEI mission is to conduct basic and applied research to enable cost-effective conversion of lignocellulosic biomass into biofuels and bioproducts. The goal of this project, performed in the Microfluidic Assays group in the Technology Division at JBEI, is to develop a robust and easy-to-use droplet microfluidic platform to automate the steps involved in engineering of metabolic pathways to produce biofuel molecules.

Synthetic biology offers a promising approach to produce biofuel and other chemicals. Optimization of metabolic pathways however, requires conducting a large number of experiments that are labor-intensive with repetitive pipetting and plating and require large amounts of expensive reagents. Robotic liquid handling stations represent a solution to automate genetic engineering processes. However, they still require a large volume of reagents and their high equipment and maintenance cost can be prohibitive to many users. Microfluidic platforms offer a promising alternative as they provide improvement over their macroscale counterparts in cost, amounts of reagents required, speed, and integration.

We are developing microfluidic devices for biofuel research applications including enzyme screening, enzyme evolution, and optimization of metabolic pathways. Our droplet-based microfluidic platforms use digital microfluidic (DMF) format where nanoliter aqueous droplets suspended in oil are manipulated on an electrode array using electrowetting on dielectric concept.[1-5] The systems can handle large numbers of droplets at once as well as actively manipulate droplets in a programmable manner, and are capable of multiple steps of droplet manipulation including formation of aqueous droplets and encapsulation of reagents and cells, electric-field driven merge and split of the droplets to add or remove liquid, on-chip electroporation, and incubation steps with localized temperature control. The device uses an array format with 100 elements, each containing sets of electrodes for two electric field actuated operations- electrowetting for merging droplets and electroporation for transformation. Reagents are introduced into the chip by dispensing droplets, are kept separate until ready to mix, mixed on-demand by merging droplets by electrowetting, and transformation of cells by on-chip electroporation.

A novel platform for high throughput electroporation is being developed in an automatable format. A 384 well microtiter plate having a novel electrode geometry is subjected to PEGylated Thiol-

Au chemistry to render the wells hydrophilic. They have individually addressable electrodes to improve the electric field transport for introducing the recombinant DNA into *E. coli* [6] as a part of the CRISPR-based MAGE as an example of how our microfluidic platform provides an alternative solution to the cumbersome traditional methods. Furthermore, with our platform, recovery, incubation and screening can be performed on the same chip. The configuration of the chip uses a 384-well template and is easily integrable with liquid handling robots. We validate our microfluidic chip by performing targeted genomic changes through CRISPR-based MAGE (CRMAGE) recombineering for the biosynthetic pathway producing the sustainable pigment indigoidine in *E. coli*.[7] The automated platform for multiplexed transformation holds the promise of accelerating the design-build-test-learn cycle.[8,9]

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