

An Automated Microfluidic System for On-Chip Genetic Engineering Processes

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Project Goals: The JBEI mission is to conduct basic and applied research to enable economically-viable conversion of lignocellulosic biomass into biofuels to provide the nation with clean, renewable transportation fuels identical to gasoline, diesel, and jet fuel. The goal of this project, performed in the Microfluidic Assays group in the Technology Division at JBEI, is to deliver robust and easy-to-use microfluidic platforms to automate the genetic engineering processes for advancing synthetic biology applications including biofuels development.

In recent years, synthetic biology has drawn significant interest for both scientific research and industrial applications, such as biofuel and pharmaceutical production. Synthetic biology processes require multiple molecular biology steps, making it a very time-consuming and labor-intensive effort. Therefore, automated and efficient processes to perform molecular biology assays are highly desired. Droplet based microfluidic technologies offer powerful approaches to improve synthetic biology processes due to faster reactions fostered by minute dimensions, reduced reagent consumption resulting from smaller working volumes, and increased control over experimental conditions.

We are involved in developing innovative microfluidic assays and integrated devices for many biofuel research applications including enzyme screening, enzyme evolution, and synthetic biology. Our hybrid microfluidic platforms utilize continuous-flow (analog) microfluidics that manipulate droplets by controlling the hydrodynamic force, and digital microfluidics (DMF) that utilize surface tension from electrowetting on dielectric with arrayed electrodes. The systems can handle large numbers of droplets at once and actively manipulate target droplets in a programmable manner. Hybrid and DMF devices are capable of multiple droplet manipulation steps including formation of aqueous droplets, encapsulation of reagents and cells, hydrodynamic capture, arraying of droplets, electric-field driven merging and splitting of droplets to achieve specific

volumes and concentrations of various reagents, on-chip electroporation, and incubation with localized temperature control.

Specifically, for electroporation devices, multiple pairs of electrodes are designed and placed at each chamber to apply voltages to arrayed droplets for on-chip electroporation. This configuration allows us to customize the electroporation conditions at each droplet for multiplexed DNA transformation processes, and it also enables us to easily scale-up the numbers of reactions for high-throughput transformation processes simply by scaling the array size. In addition, we integrate optical fibers in the microchannels to add on-chip fluorescence-based detection of encapsulated cells and enzymatic activities in the discrete droplets and for triggering sorting of droplets. We utilize our microfluidic methodologies for automating CRISPR/Cas9 based gene editing processes such as recently the established CRMAGE for *Escherichia coli* or the cloning-free tool kit for *Saccharomyces cerevisiae*.

Unlike conventional microtiter plate-based reactions, our analog-digital microfluidic platforms with on-chip electroporation and fluorescence detection allow completely automated genetic engineering steps using 10-100-fold lower amounts of reagents and can be useful for applications requiring high throughput screening and reactions.

References

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