

An Automated Microfluidic System for Gene Editing 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 the 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 dramatically grown and became significantly important for both of scientific researches and industrial applications such as biofuel and pharmaceutical applications. However, multiple genetic engineering steps required for synthetic biology are often time-consuming and labor-intensive with repetitive pipetting and plating. Therefore, automated and efficient processes to perform molecular biology assays have been long desired. Microfluidic assays and devices with aqueous droplets (microliter to picoliter in volume) suspended in oil phase as compartmentalized reaction chambers have attracted a significant attention for performing biochemical reactions and analysis as they provide drastic improvements over their macroscale counterparts with various benefits such as faster reaction time, less volume of reagent consumption required, better control of experimental environment, and higher throughput with multiplexed processes.

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 the 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 as well as actively manipulate target 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, hydrodynamic

capture and array of the droplets, electric-field driven merge and split of the droplets to add specific amount and concentrations of various reagents, on-chip electroporation, and incubation process with localized temperature control. Specifically, multiple pairs of electrodes are designed and placed at each chambers to apply voltages to the arrayed droplets for on-chip electroporation. This configuration allows us to modify and program 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 designing the same structures in an array. In addition, we integrate optical fibers in the microchannels to add on-chip capability for 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 established CRMAGE for *Escherichia coli* or 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 application requiring high throughput screening and reactions.

References

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