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 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 tiny (nL) 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.

One example platform is a device for multiplexed electroporation for automating CRISPR-based MAGE recombineering in *E. coli*.[6] 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 transform cells by on-chip electroporation. Additional reservoirs allow recovery incubation and screening on 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]

References

1. P.C. Gach, K. Iwai, P.W. Kim, N.J. Hillson, and A.K. Singh, "Droplet Microfluidics for Synthetic Biology," *Lab Chip*, 2017, **17**, 3388-3400.
2. P.C. Gach, S.C.C. Shih, J. Sustarich, J.D. Keasling, N.J. Hillson, P.D. Adams and A.K. Singh, "A Droplet Microfluidic Platform for Automating Genetic Engineering," *ACS Synth. Biol.*, 2016, **5**, 426-433.
3. S.C.C. Sigh, G. Goyal, P.W. Kim, N. Koutsoubelis, J.D. Keasling, P.D. Adams, N.J. Hillson and A.K. Singh, "A Versatile Microfluidic Device for Automating Synthetic Biology," *ACS Synth. Biol.*, 2015, **4**, 1151-1164.
4. S.C.C. Shih, P.C. Gach, J. Sustarich, B.A. Simmons, P.D. Adams, S. Singh and A.K. Singh, "A Droplet-to-Digital (D2D) Microfluidic Device for Single Cell Assays," *Lab Chip*, 2015, **15**, 225-236.
5. P.C. Gach, S.C.C. Shih, J. Sustarich, J.D. Keasling, N.J. Hillson, P.D. Adams and A.K. Singh, "A Droplet Microfluidic Platform for Automating Genetic Engineering," *ACS Synth. Biol.*, 2016, **5**, 426-433.
6. C. Ronda, L. E. Pedersen, M. O. Sommer, and A. T. Nielsen, "CRMAGE: CRISPR Optimized MAGE Recombineering", *Sci. Rep.*, 2016, **6**, 19452.
7. M. Wehrs, J.M. Gladden, Y. Liu,ac Lukas Platz, J.P. Prahl, J. Moon, G. Papa, E. Sundstrom, G.M. Geiselman, D. Tanjore, J.D. Keasling, T.R. Pray, B.A. Simmons and A. Mukhopadhyay, "Sustainable bioproduction of the blue pigment indigoidine: Expanding the range of heterologous products in *R. toruloides* to include non-ribosomal peptides", *Green Chem.*, 2019, **21**, 3394-3406.
8. T. Radivojević, Z. Costello, K. Workman, and H.G. Martin, (2019) ART: A machine learning Automated Recommendation Tool for synthetic biology. *arXiv*.
9. R.D. King, K.E. Whelan, F.M. Jones, P.G.K. Reiser, C.H. Bryant, S.H. Muggleton, D.B. Kell, and S.G. Oliver, (2004) Functional genomic hypothesis generation and experimentation by a robot scientist. *Nature 427*, 247–252.

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