

# Cell-Free Transcription and Translation Reactions with Droplet Microfluidics

Ali Lashkaripour, Luis Ortiz, Marilene Pavan, Radhakrishna Sanka and Douglas Densmore

CIDAR Lab, BioDesign Center, Department of Electrical and Computer Engineering, Boston University, Boston, MA

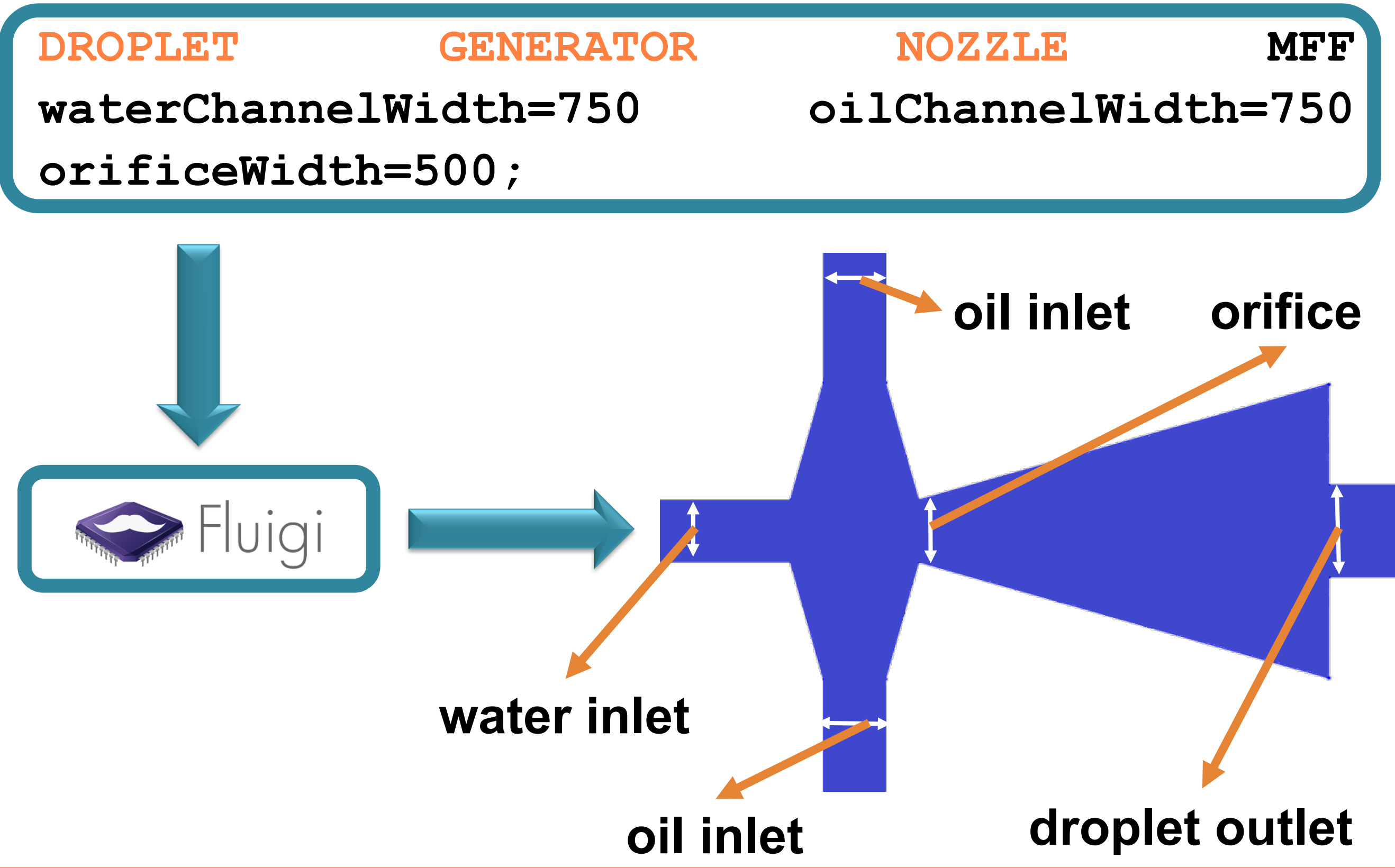


## Abstract

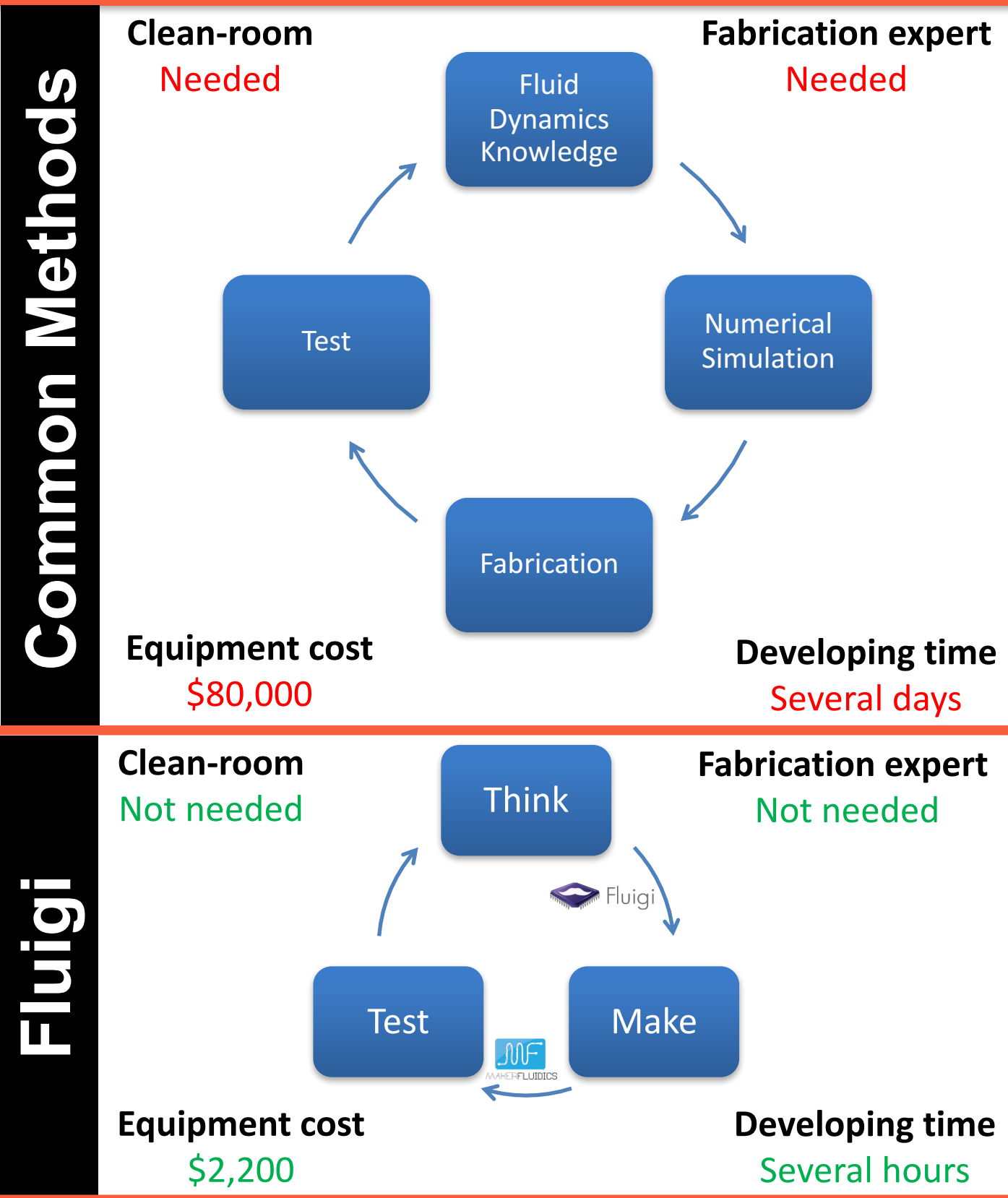
Cell-free systems have become a powerful tool in bioengineering and synthetic biology. Their high throughput and increased expression in comparison to *in vivo* conditions made it especially desirable for biological network prototyping and protein engineering. We used our lab's novel, automated CAD tool to design microfluidic chips and a fast fabrication process, that combined together enables us to do inexpensive (less than \$1) and rapid prototyping (less than 1 hour) of microfluidic chips. Through this workflow which we call Fluigi, we were able to design and fabricate a microfluidic chip that is able to carry out cell-free transcription and translation (TX-TL) reactions inside water-in-oil droplets.

## Automated Microfluidic Design

Fluigi is a fully automated CAD tool, that designs microfluidic chips based on high level specification. This tool is able to design large scale and complex microfluidic chips. The Droplet Generator is one among many other microfluidic primitives embedded in Fluigi. By few lines of code you can automatically design the Droplet Generator by defining the feature dimensions. Fluigi will later on place and route the Droplet Generator to connect to the rest of the microfluidic chip. Unlike common CAD tools, to change the Droplet Generator dimensions only three numbers should be altered.



## Inexpensive Rapid Prototyping of Droplet-Based Microfluidic Chips



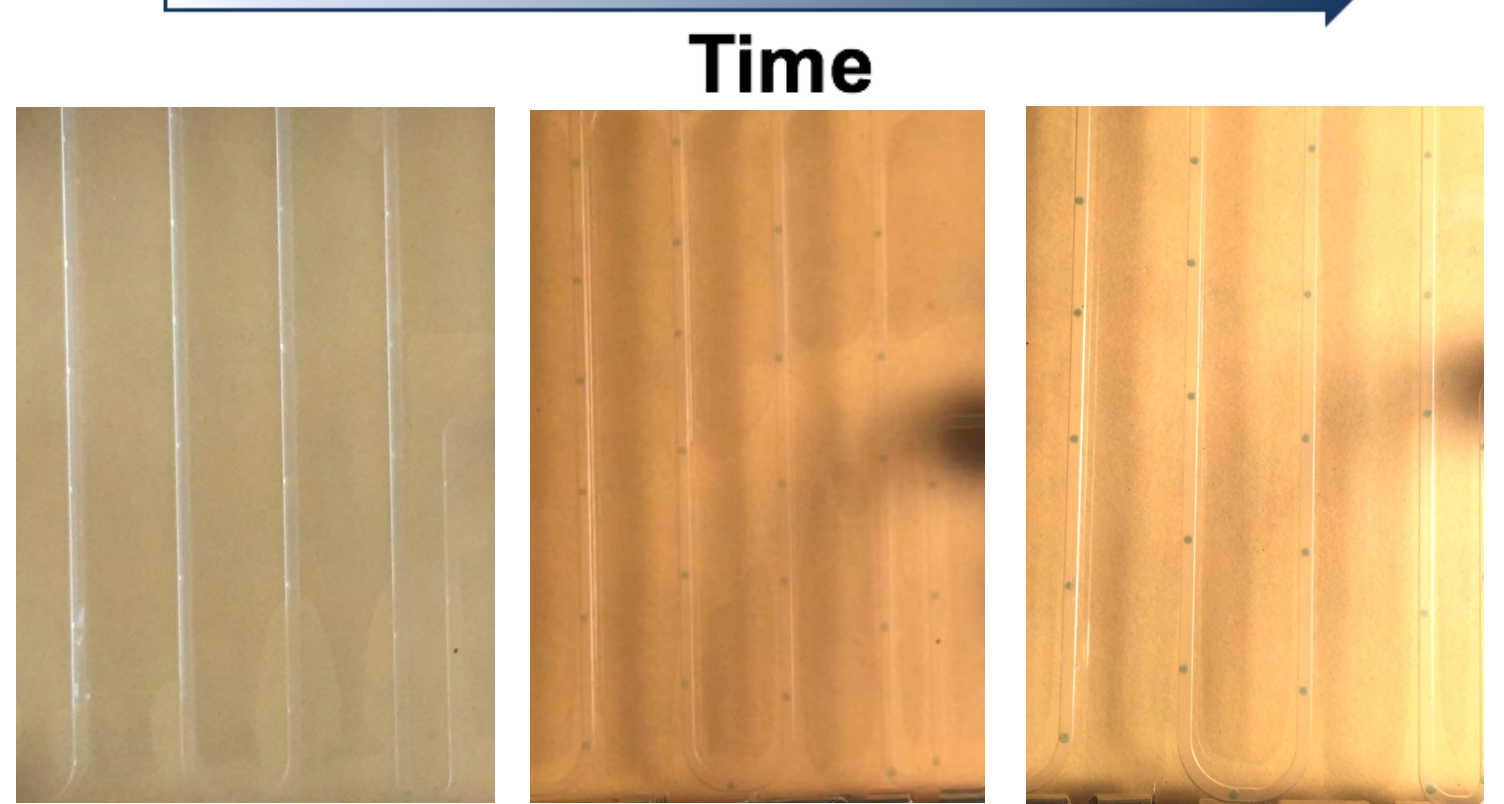
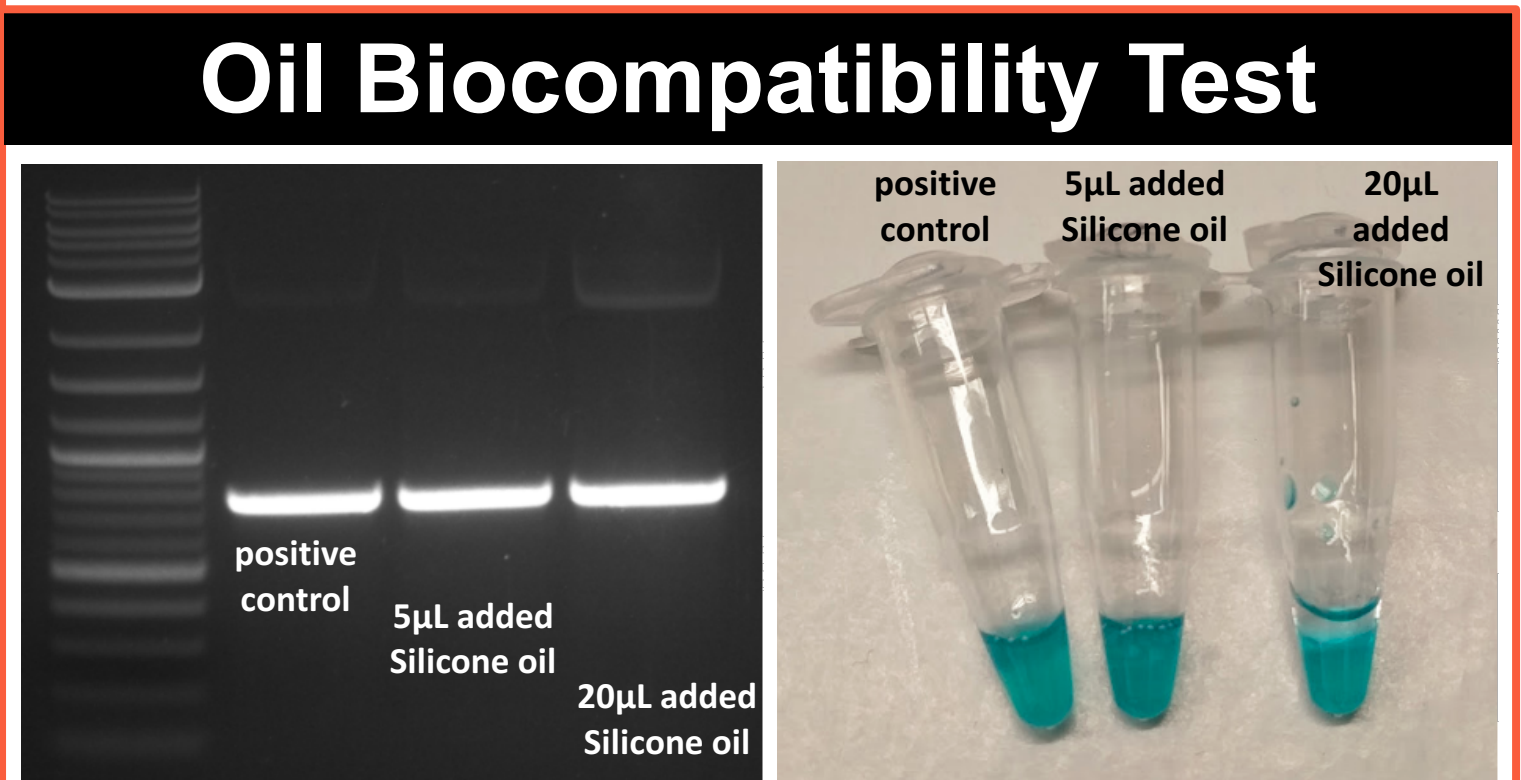
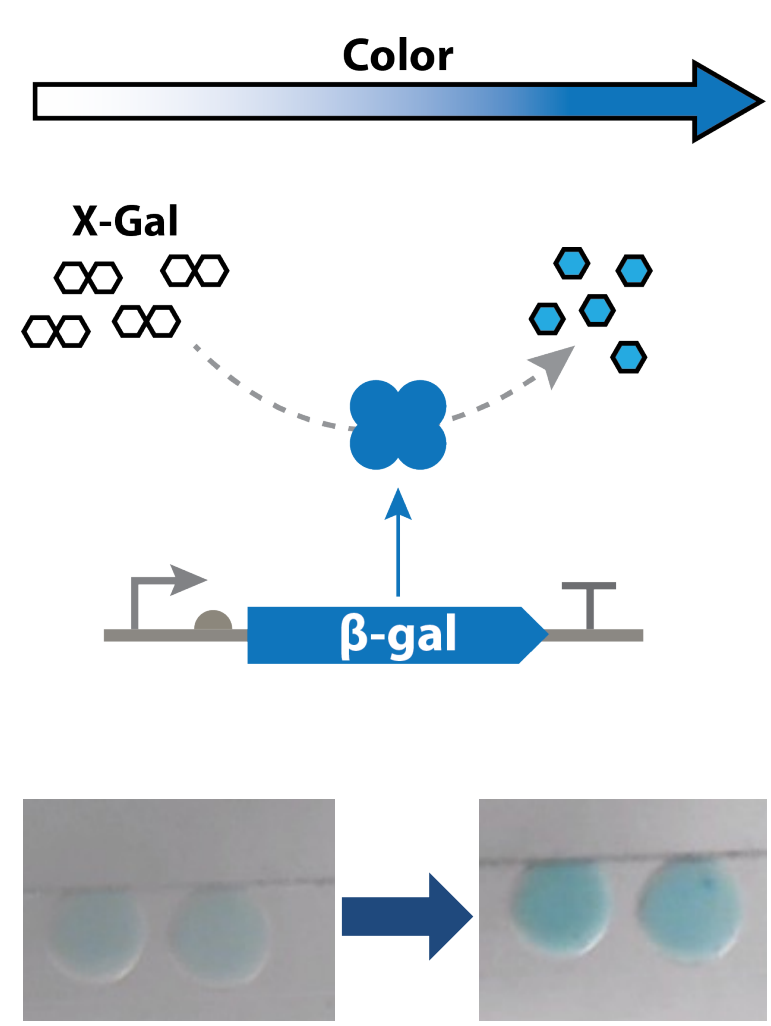
MakerFluidics is a low cost fabrication method that uses a desktop CNC mill to cut out geometries. This CNC takes the output of Fluigi and fabricate it in less than an hour. Through this workflow we were able to develop droplet based microfluidic chips rapidly and cheaply in a polycarbonate chip.

Microfluidic Chip	Droplet Generator	Droplet Incubator	Droplet Merger	Droplet Mixer

Once a design is verified, it could be easily integrated with other microfluidic primitives such as Y-mixer, channels, valves and ports, to expand the microfluidic chip complexity for various applications.

## Cell-Free TX-TL with Droplet Microfluidics

We developed a cell-free system, compromised of purified E. Coli cytoplasmic extract. This system with added DNA and X-Gal, was implemented inside a droplet-based microfluidic chip. The DNA added to our cell-free mix is an expression plasmid driving  $\beta$ -galactosidase, an enzyme which cleaves the substrate X-Gal to produce a blue pigment. Thus,  $\beta$ -galactosidase will be expressed which will cleave X-Gal produce a blue colorimetric readout in droplets. Therefore, color change verifies the success of our cell-free system.



## Conclusion and Future Work

Fluigi and MakerFluidics together provide an inexpensive rapid prototyping tool for microfluidics. Through this workflow we designed a droplet based microfluidic chip that carries out cell-free TX-TL reactions inside water-in-oil droplets. This microfluidic cell-free system can be further expanded for various applications including protein engineering and directed evolution.

## References

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- [2] Huang, H. et al. "Integration of microfluidics into the synthetic biology design flow", Lab on a Chip, vol. 14, iss. 18, pp. 3459-3474, 2014.

## Acknowledgments

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