DIRECTED EVOLUTION FOR BIOSENSOR CREATION

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BACKGROUND

Molecular tools for assaying complex environmental changes, are require complex engineering. Directed evolution has played a vital role in this, with a constant push to engineer faster evolution, at greater scales with ease of use making it optimal for controlled selection of a mutation in bacteria [1]. Developing directed evolution for biosensor engineering will require continuous turbidostatic culture, phage assisted methods, and both parallel positive and negative selection. To meet this challenge, the researchers adopted an open source continuous cell culture platform (eVOLVER) and designed new features for increased control of evolutionary parameters. The eVOLVER is currently under construction and will be optimized for the multiplexed selection conditions needed for biosensor engineering.

RESEARCH METHODS & EXPERIMENTAL DESIGN

To develop a continuous turbidostatic culture feed to supply the directed evolution system, OD_{900} was used to measure the turbidity of the media, and a feedback loop was created to allow for controlled media addition and removal to maintain constant turbidity.

The directed evolution setup was modeled after the standard eVOLVER setup, however where the continuous culture is pumped into lagoons which the additional influx of chemical inducers to activate mutagenesis. Under positive selection conditions additional influx channels for inducers are included Luminescence data is also collected used to measure the sensor on state.

To select for off states, outflow to a second lagoon is added, with influx channels for negative selection inducers and feedback from luminescence.

REFERENCES

[1] Cobb, R. E., Chao, R., & Zhao, H. (2013). Directed Evolution: Past, Present and Future. *AIChE journal. American Institute of Chemical Engineers*, *59*(5), 1432–1440. https://doi.org/10.1002/aic.13995

[2] Brandon G Wong et al. "Precise, Automated Control of Conditions for High-Throughput Growth of Yeast and Bacteria with eVOLVER." *Nature Biotechnology* 36.7 (2018): 614–623. Web.

RESEARCH QUESTION

How can we automate directed evolution to develop dynamic biological sensors?

RESULTS

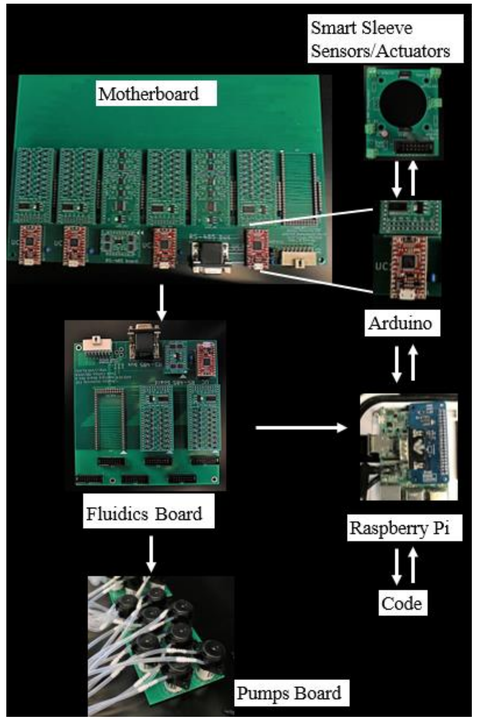

Figure 2: Signal transfer (arrows) in the directed evolution eVOLVER system is shown in relation to the hardware. The Raspberry Pi receives software signals and communicates with Arduinos to record and modify experimental conditions in turbidity, stir rate, luminescence, and temperature. The fluidics system controlling smart sleeve fluid influx/efflux is controlled through signaling between the motherboard and smart sleeves.

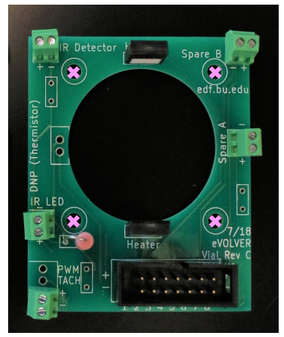

Figure 1: The vial board demonstrates the different components of the smart sleeve. Locations for the sensors and actuators for turbidity, temperature, stir rate, and luminescence are denotated by the light pink components. The center remains open to allow for aluminum sleeve and vial insertion.

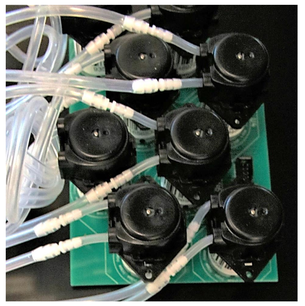

Figure 3: This pump board from the fluidics eVOLVER setup allows for smart sleeve media control through precise influx/efflux of media, controlled by peristaltic pumps.

DISCUSSION

The described system shows promise in its potential as a molecular biosensor development platform. Turbidostats have been shown to be compatible with directed evolution, which is supported by the precise cultured media input that the eVOLVER system is able to provide. Although the eVOLVER has presently only been used for continuous culture, its value in directed evolution can be seen in the control that may be exercised over the lagoons through detailed sensor/feedback systems as well as continuous parameter modifications (Figure 1). This control both in electrical sensing/actuating and fluidics as depicted in Figure 1 and Figure 3 further demonstrate eVOLVER's feasibility in two-lagoon directed evolution, as parallel selected evolution may be carefully controlled and monitored through luminescence recordings. Although the system is in development, for engineering biological sensors, its precise feedback control and modularity can be used to engineer any biological function possible in a cultured organism.

OBSTACLES

A major obstacle faced was the COVID-19 pandemic. This made working in the lab impossible and delayed our findings. This was partially overcome through consistent online communication.

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