Open Plant Funding Proposal

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Summary

One of the primary goals of synthetic biology is to use the components of biological organisms to produce valuable biochemicals from a stock of commodity substrates. In an effort to advance the progress of the field towards this goal, our team has developed a two-part project that combines the latest advancements in machine learning with microfluidics-based cell free screening assay technology. In the first part of the project, a Generative Adversarial Network (GAN) has learned how to generate a plausible reaction pathway between any two small molecule biochemicals. For each step in this pathway, a second GAN has learned to generate amino acid sequences that could plausibly catalyze each step in the reaction pathway in a cell-free environment. The second part of the project is to test the effectiveness of the algorithm *in situ* via a high efficiency open-source microfluidics device. This platform must be capable of expressing predicted enzymes, testing them against their substrates, sampling the product solutions for input into an LC-MS, chaining reactions together into pathways, and interfacing with high-throughput laboratory automation hardware such as an OpenTrons or a LabCyte Echo.

1. Project Proposal

1.1 Components

The microfluidics system will consist of two 3D printed components housed in an automated lab-pipetting system. Each component will be structured in a standard-well plate design with modifications to support this specific application. The first component is a Cell Free Protein Synthesis plate, which will be similar to the design of a similar device described by Jackson *et al*. 2014. This will contain a chamber that houses transcriptional and translational machinery, which will be connected to a microfluidics channel feeding system that will supply ligands, amino acids, nutrients, and energy to drive enzyme production. The second component is a plate of a similar design, where each well is a reaction chamber that is fed substrates and energy in the form of ATP, etc. via microfluidics channels.

Transfer of new enzymes to a substrate conversion chamber will be done by hand, or by using an OpenTrons or LabCyte Echo. Similarly, transfer of a biochemical product from a reaction chamber to serve as the substrate for the next step in the pathway in a different reaction chamber will be done either by hand, or by using an OpenTrons or LabCyte Echo.

1.2 Materials and Assembly

The two microfluidics plates will be manufactured using a Formlabs 2 SLA printer. Other components of this system will be purchased from commercial vendors to expedite the project and to ensure reproducibility for the open-source community.

1.3 Operation and Components

The input of the system is a series of algorithmically generated sequences of oligonucleotides. The output of the system is a series of mass spectrographs. For the purposes of simplicity, in order to demonstrate the effectiveness of the system, the *de-novo* manufacture of oligonucleotides and the measurement of product will be done using commercial services. Similarly, the initial commodity substrate, the transcriptional and translational machinery, the nutrients and chemical energy components required to drive reactions will be sourced from commercial vendors. Where possible, liquid handling protocols be automated using the OpenTrons or LabCyte Echo. The OpenTrons protocols for the use of these devices will also be made open source.

1.4 Outcomes

The result of this project will be an open source SLA printable system for high-throughput screening of cell-free enzymes and enzyme activity. This system will be tested and documented on Read-The-Docs, and all files will be hosted in an open GitHub repository. We are exploring the possibility of adding this project to DocuBricks.

2. Team

Clayton Rabideau

A PhD Candidate in the University of Cambridge department of Chemical Engineering and Biotechnology, who has previously worked to improve reproducibility in the Microbial Fuel Cell (MFC) field by creating a 3D printed/Printed Circuit Board hybrid well-plate style MFC array. Has worked with various groups in the Computer Sciences department to develop the sequence generator algorithm.

Stefan Grossfurthner

First year PhD student in the Smith group at the Department of Plant Sciences and funded by the BBSRC Doctoral Training Partnership. Working on the application of synthetic biology to metabolic engineering of microalgae for production of high-value products.

3. Allocation of Resources

The vast majority of the £4000 will be allocated towards the consumables listed in the table below. We have access to a Formlabs 2 3D printer already.

Bill of Materials

Item	Cost Per Unit	No. of Units	Sub-Total
Resin Cartridge	£143	2	**£286**
Resin Tank	£59	1	**£59**
Printer Depreciation / Hour	£1	100	**£100**
Peristaltic Pump 1 (Temperature Control)	£20	10	**£200**
Peristaltic Pump 2 (Microfluidics Control)	£400	1	**£400**
Cell-Free Expression Kit	£475	2	**£950**
1kb De-Novo gene synthesis	£260	5	**£1300**
LC-MS Analysis / Sample	£75	5	**£375**
Raspberry Pi 3 Model B	£32	1	**£32**
GRAND TOTAL			**£3702**