**Microfluidics for Control of Synthetic Biology**

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Microfluidic systems with integrated analysis and control of environmental parameters can enable precise control of metabolic activity and protein expression. Here, we couple microfluidic controls in a polycarbonate-PDMS microbioreactor (Fig. 1) with closed-loop control of temperature, pH, dissolved oxygen, and cell density [1,2] to the biochemical control of synthetic gene circuits [3,4]. In situ measurements of optical density were used to calibrate flow rates and peristaltic mixing times in the microbioreactor growth chamber. Long-term culture requires precise injection and extraction of fluid from the 1 mL growth chamber. In this case, 831.3 nL injections were used for fluid addition/extraction via peristaltic metering valves [2]. This precision comes at the cost of slow change-over times for the chemical concentration (here glucose and galactose) in the growth chamber – the measured change-over time was around 6 hours for flow rate typical for yeast culture. However, a control algorithm can be used to dynamically alter the concentration of the injected glucose/galactose to rapidly reduce the change-over time to <1 hour achieving control of the chemical environment faster than the doubling time (Fig. 2).

A Saccharomyces Cerevisiae strain with dual inducible reporter proteins was constructed for this study. The construct allows for induction of either red fluorescent protein (RFP) or green fluorescent protein (GFP) depending on the carbon source used for growth. As illustrated in Fig. 3, expression of RFP is induced in the presence of galactose but repressed in glucose, while GFP is only expressed in the presence of glucose and aTc (a small molecule inducer). In order to test the galactose induced expression of RFP, continuous turbidostat culture (where the microbioreactor automatically adjusts the flow rate to maintain a constant cell density in log phase) with glucose and galactose in the input medium toggled in the microbioreactor (Fig. 4). Samples were extracted from the microbioreactor after steady-state was reached (at 24-hours), after the carbon source was switched from glucose to galactose with dynamic control of injected concentration (48-hours), and after the carbon source was switched back to glucose (72-hours). Flow cytometry histograms in Fig. 5 indicate the RFP level for samples taken from the microbioreactor and from a comparison experiment with test tube cultivation on the same strain with 24 hours as the induction period. As can be seen from the histograms, the fold induction for the microbioreactor (~400x) is much higher compared to the test tube (~50x). The better induction performance is likely due to the microbioreactor’s ability to supply nutrients continuously. After switching the carbon source from galactose back to glucose, the RFP induction is suppressed to its base level. This represents complete and reversible on-and-off switch with microfluidic control over synthetic gene circuits.

This data suggests that the protein expression throughout the cell population is more uniform as can be observed from the tighter induction distribution from the microbioreactor compared to the test-tube cultivation. With the much-improved and better-characterized synthetic gene circuit performance under controlled cultivation, our device creates new avenues for controlling, characterizing, and standardizing synthetic biology parts.

REFERENCES:
Fig. 1. A schematic diagram showing the plastic-PDMS culture chip configuration used in this study. The actual device photo is shown on the top left side of the figure.

Fig. 2. Flow calibration experiment with online optical sensor showing a pre-emphasis microfluidic injection control for rapid medium change-over under constant flow rate conditions typical for yeast cell culture. The input medium has two times higher concentration compared to the target concentration in this case.

Fig. 3. A schematic diagram showing the two-input and two-output synthetic gene circuit used in this study. pGAL1 is a galactose inducible promoter. pMinCYC-Tet is a glucose and aTc inducible promoter. RFP and GFP are the reporter proteins for these two promoters.

Fig. 4. The optical density, dissolved oxygen, and flow rate (equivalently growth rate under turbidostat) plot for the microbioreractor cultivation. The temperature is controlled within 30±0.5 °C and the pH is self-maintained within 6.7±0.2 throughout the entire cultivation period. The pre-emphasis injection control shown in Fig. 2 is used for rapid medium switching. The flow cytometry histograms showing the RFP induction level at steady-state (24, 48, and 72-hours before medium switching) are plotted alongside the OD plot. Over ~400x fold induction is observed from glucose-to-galactose switching. Complete suppression of induction is achieved by switching the carbon source back to glucose.

Fig. 5. Flow cytometry histograms showing the comparison of RFP induction levels between test tube and microbioreractor cultivations. The microbioreractor cultivation shows much higher fold induction as well as much tighter induction distribution compared to the test tube cultivation, indicating the important implication of controlled environment over synthetic gene circuit performance.