

Supplementary data for the article:

Ostafe, R.; Prodanovic, R.; Ung, W. L.; Weitz, D. A.; Fischer, R. A High-Throughput Cellulase Screening System Based on Droplet Microfluidics. *Biomicrofluidics* **2014**, 8 (4). <https://doi.org/10.1063/1.4886771>

Supplemental Material

Title:

A high-throughput cellulase screening system based on droplet microfluidics

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Experimental data

Fabrication of microfluidic devices.

PDMS devices were prepared using soft lithography as previously described^[8].

Emulsification and sorting.

Monodisperse aqueous drops in fluorocarbon oil (Novec HFE 7500, 3M, Minnesota, USA) were prepared using a co-flow, flow-focusing geometry. The fluorocarbon oil contained 1% (v/v) Krytox-PEG-Krytox detergent synthesized as previously described^[11]. Two solutions were co-flowed, one containing library cells (*S. cerevisiae* YPH500 cells, Agilent Technologies, Santa Clara, USA) and the other containing the substrate. The library cells comprised a defined mixture of cells transformed with cel5A pESC-Trp (positive cells) or empty pESC-Trp (negative cells). The substrate solution consisted of APF (100 μ M, Invitrogen), VBrPOx (200 mU/mL, Sigma Aldrich), HOx (3 U/mL, Danisco Deutschland GmbH), CMC (0.3% (w/v), Sigma Aldrich), NaBr (10 mM) and BSA (1.5 mg/mL) in Tris H₂SO₄ (50 mM, pH 7.4). We obtained 30- μ m drops by flowing the three streams at 15 μ Lh⁻¹ (cells and substrate stream) and 30 μ Lh⁻¹ (oil stream), respectively, through a 10- μ m nozzle. The drops were passed through a 20-min incubation line and re-injected into a sorting device, where the positive drops were sorted as previously described^[8] onto YNB CAA Glu agar plates. The plates were incubated at 30°C for 2 days.

Agar plate assay.

Colonies were transferred to YNB CAA Gal/Raf 0.1% (w/v) CMC plates for the agar plate assay. The YNB CAA Gal/Raf 0.1% (w/v) CMC plates were incubated for 24 h at 30°C and the Congo red agar plate assay was performed as previously described^{[5], [13]}. Cells expressing cellulase activity were revealed by transparent halos.