Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

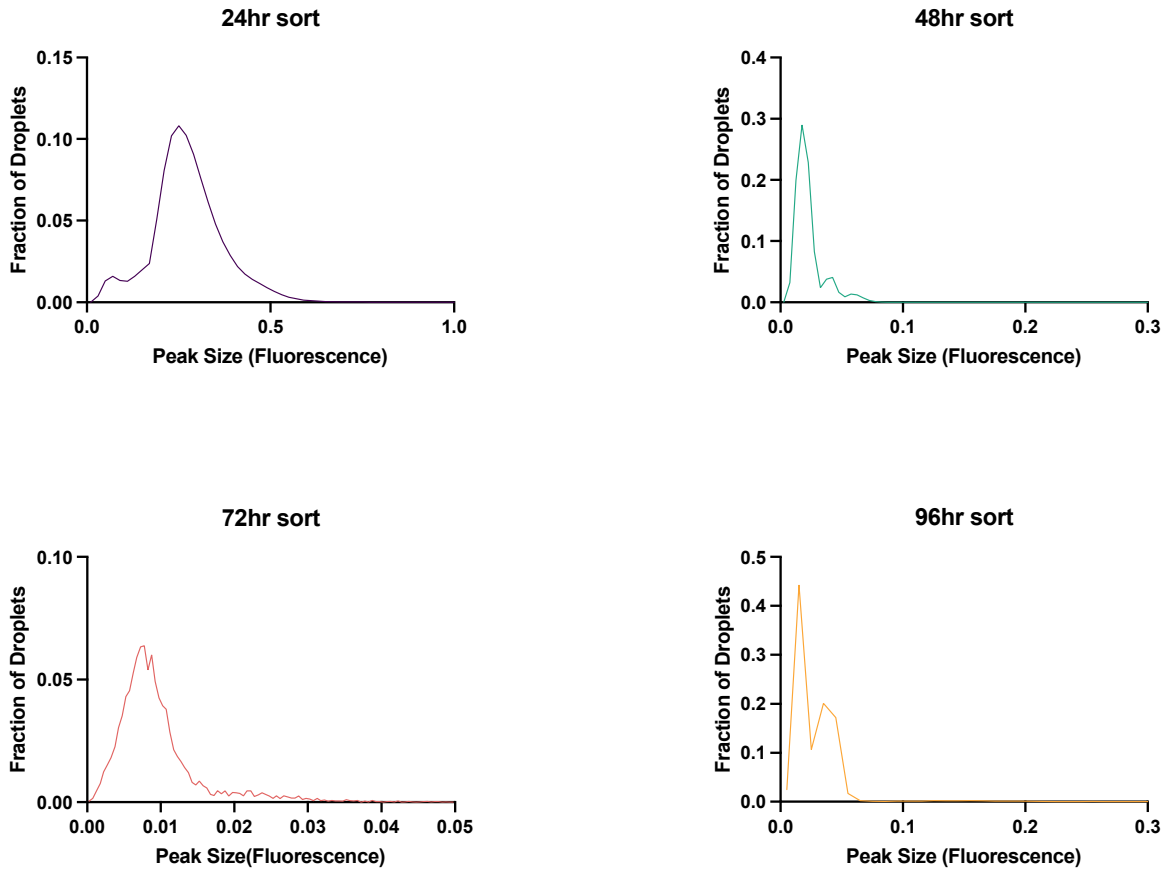
Table of Contents:

Supplementary Figures:

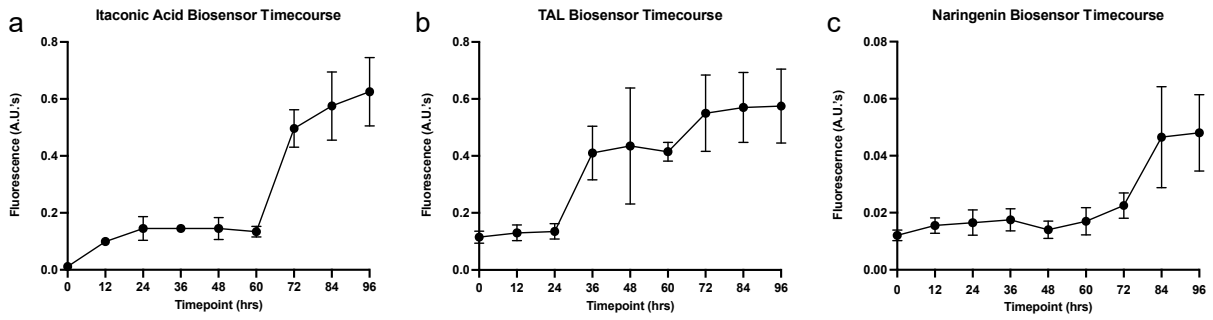
Supplementary Tables:

Supplementary Appendix:

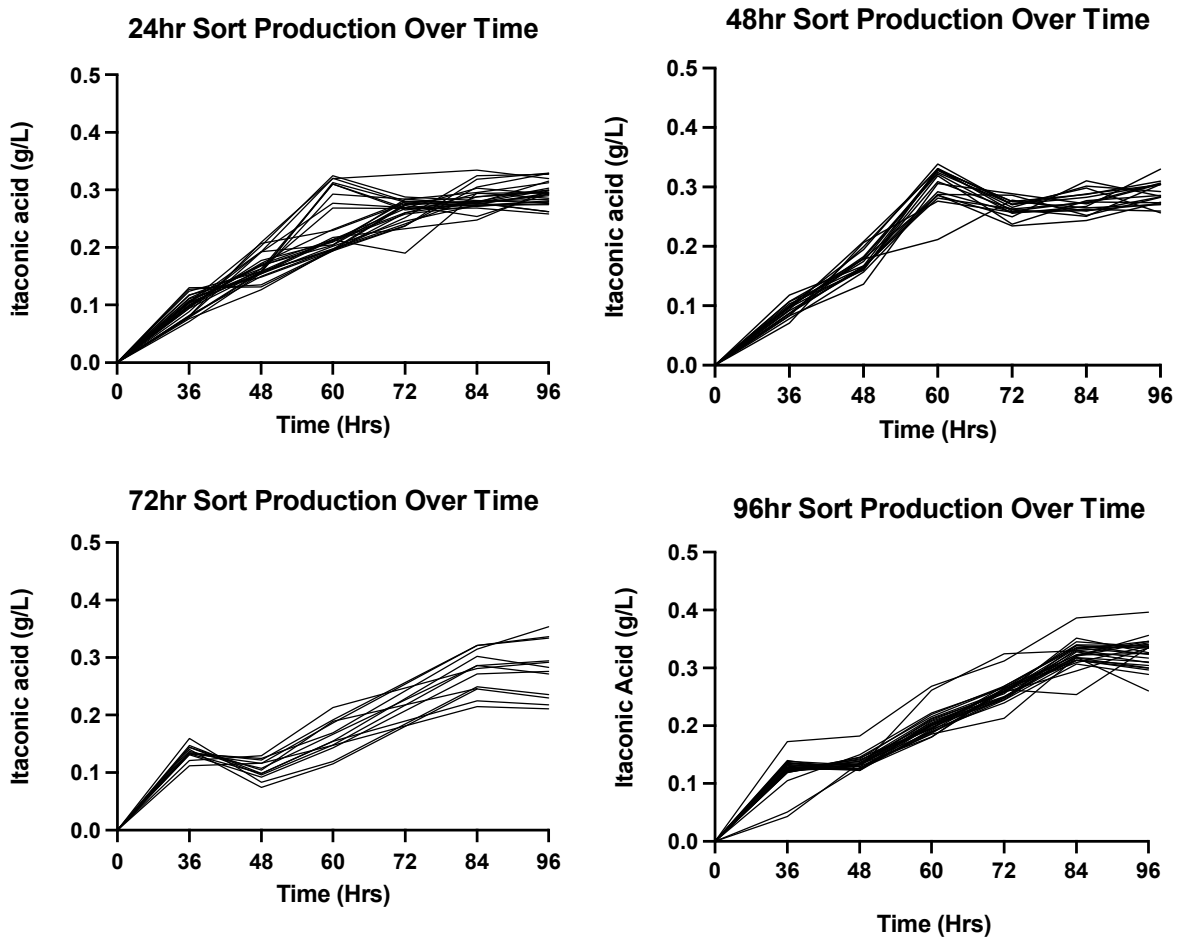
Supplementary Figures:



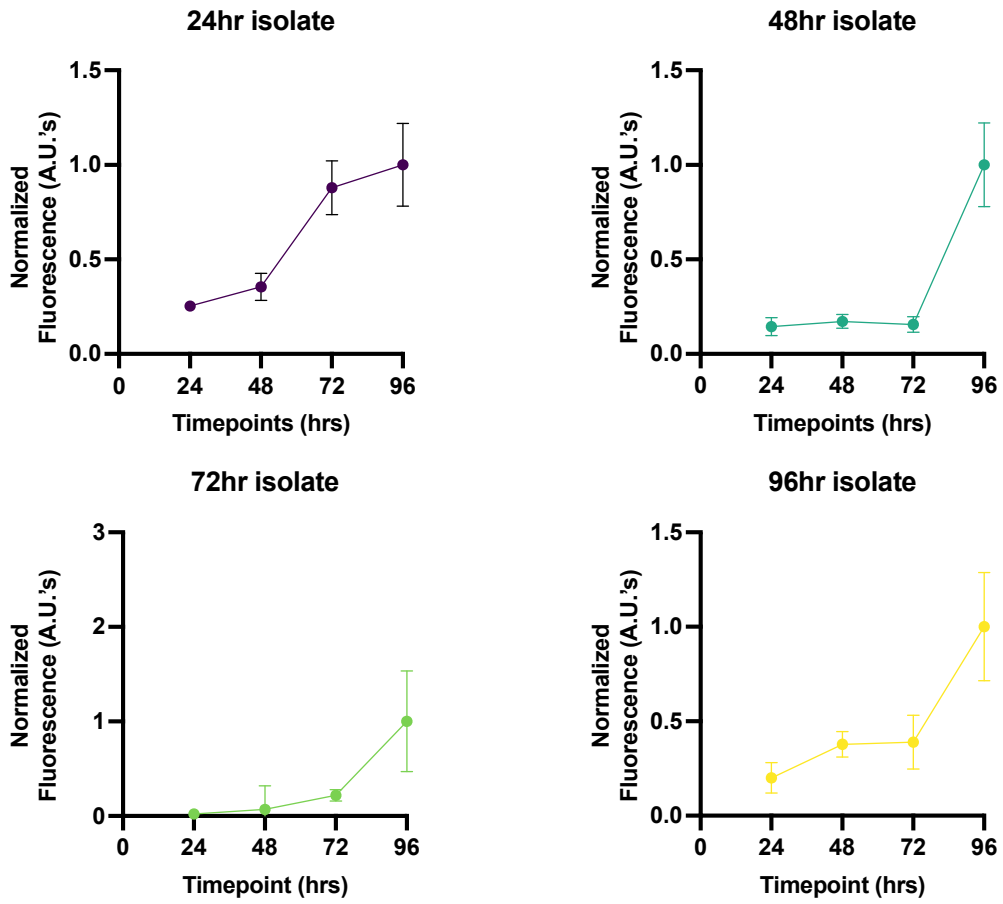
Supplementary Figure 1: *Time course sorting for distinct production phenotype isolation.* Emulsions were generated and incubated at 28°C at 24-hour intervals. Sorts were carried out after their corresponding incubation times, and the top 10% of droplets were collected based on overall fluorescence, graphed here. Peak size/fluorescence are not comparable sample-to-sample as gain and laser strength were adjusted to account for signal strength. The 24-hour sample had to be iteratively sorted for a total of two sorts in order to achieve statistically significant differences between the unsorted and sorted population. This histogram represents that collected from the second sort, thus is not a full representation of an unsorted library at 24 hours.



Supplementary Figure 2: *Response curves of each biosensor at 12 hour timepoints.* Previously-characterized production strains of *Y. lipolytica* were co-encapsulated with corresponding *E. coli* biosensors for the production of Itaconic Acid (a), Triacetic acid lactone(b), and Naringenin(c). Emulsions were subsequently incubated and run on the droplet sorting instrument (Sphere Fluidics) to collect fluorescence information from droplets at each time point. droplet loading was 0.1 cells/droplet for *Y. lipolytica* and 10 cells/droplet for *E. coli* to reduce co-encapsulation of producers as modeled via the *Poisson distribution.* Therefore, only the top 10% of droplets represent those containing both the producer strain as well as the biosensor, resulting in a dose-based response.



Supplementary Figure 3: *Production Curves of Populations from Each Sort.* Top performing isolates from each sort were cultured for a total of 96 hours with itaconic acid production and OD sampled every 12 hours. a) Production curves of the 24- and 48-hour isolated population over time. Early sort timepoints (24 and 48) show a clear peak in production at the 60-hour timepoint followed by a plateau. b) Isolates sorted at late timepoints (72 and 96) show a slow increase in production that peaks at the 84-hour timepoint.



Supplementary Figure 4: *Fluorescent curves obtained from microdroplet encapsulation of top performing isolates.* Best-performing isolates from each sort were re-encapsulated with itaconic acid biosensor. Fluorescent measurements were obtained via our FADS system and plotted.

Supplementary Tables:

Primer Name	Sequence
CP215	AGATGTCCTAAATGCACAGCGAC
CP216	CTCCAAGCGGCGACTGAG
CP217	CATTGACAAGCACGCCTCAC
CP218	CTCGATATACAGACCGATAAAACAC
CP219	CATGATTATCTTTAACGTACGTCACA
CP220	NTGCANTNTGCNGTT
CP221	NGTCAGNNNGANANGAA
CP222	NGTGNGANANCANCANAG
CP223	TGNGNGANANCANAG
CP224	AGNGNAGNANCANAGC

Supplementary Table 1: *Primers used for TAIL PCR of isolate Piggybac insertions.* Nested primers (CP215-219) were identified from Fonager et. al, and Liu et. al (25, 26). Degenerate primers (CP220-224) were identified from Kalyani et. al, (27).

Supplementary Data 1: *Sequencing data of gene perturbations from TAIL PCR.*
Example sequences identified via TAIL PCR and SANGER sequencing. 3' PiggyBac ITR highlighted in pink.

Ygsy1 **perturbation:**

GAAAATGCTCCCCAATCTGCCGCCACGAAACTGTAATGCAATATGATGCCAATCGTGTATGGTAACTG
ATTATGCAGCTCATCACGGCTCCTATAAAGGCAACAGCTCCCAAATTCACAACAAGGCGGCTTTCAATG
CATCATGGTGTGGTATGTCTAAGTCATCAAGGATCGTTCAATTATTGACGGTAGCTAGATCACTGATGC
ATTCCATACGAGGAAGACGTCCCCAGCGCCAGAGAAAGAGAGACTGGTATGGAGCTGGGTCATTATG
GGTAGACTCTAAGAGCTCCTGCCAGGTTGCAGGCTCTGGGGAAGGGCATTTGAAGAACATGTCTAAG
TCCAAGTTGTCCAGAATGCCCAACAGAATATCAGACTAAAAATCTGAAGAGTCAATACCGCCAGAATC
CATGGGAGATGCTCTGGAGGGGTGACAACAGGGCCTGCGCCTGCTGCGGACTCAGCAGACCCGGCCA
CTGGCCTCACTTCATTTCCCTGGGCTTCCGCCTCCTGTTCAGCAACCAGAGCATCCATCCGCAAGCGCTG
TCCAACTCCTGGTTCTCAACTACATGACCAACAGTTTTCTCGAGTAAAAGGTGATTTTGTAGCAAAAGT
CTAAGGTTATCATCTTCTCAATCTTCCACGAGATGTTCCAGCTCATTCATTCGAGCAATCGTTACATCTA
GGGCAGTATGATATGGTATTTTTATTATTTTTTAACCAGCGGCGACTGAGGACCCGAAAGCGAGGAGC
CACTGGAGGAATCATTCTCACCGCCTCCCACAACCCCGGAGGACCCACCGAGGACTTTGGAATCAAGT
ACAACCTCGCCAATGGCGGGCCCGCTCCCGAGTCCGTTACCAACAATATTTTCGACGTGACCACCAAG
ATCAAGGAATACCAGCGAGTCGACGTGGGAGAGGTCGACCTCAAGACCGTCGGCACCAAGCAGTACG
GTCCCATTGAGATTGAGATCATTGACTCTGCCGCCGACTACGTCGCTATGATCAAGGATATCTTCGACC
TGGAGCTCATCAAGGACTTCCTCAAGTCCAACCCTGACTTCAAGGTCCTCTTTGACGGTCTTAACCCTA
GAAAGATAGTCTGCGTAAAATTGACGCATGCATTCTTGAAATATGCTCTCTCTCGATATGCCATGC

Ypgm1 **perturbation:**
NNNNCGGTGTGGAATTCCGNACAAGATNCTAGGAAANNGNATANGNNCATGCCANCAGTGNNACG
TGGNNGCAATCGGTCGGCCTCTCGCCTATAACTGCCAGCTGNNNGGGATTNCTGCAGCGATAGNTGT
TACGNNGNTTCCAGTCACGACGTGTAACCGACGNNAGTGATCGCACATCACAGCTACGAGTCGAGCA
CTGCTCAGCGAAAGCCCGACGTGTGTGCCCACGTCTCACGTGGTCAAGTTTTCGGCATGCACGAGTTCC
AGACCTGCATGCCCTGATGAAGGACAAGATCAACCAGTTTGTCAAGGGCCACTTTTACGGTCACCTGG
ACTTTGATCTCGACGACACTCTGTATACCTTTACTGCCGGCCGATACGAGTACCGAAACAAGGGTGTA
GACATGTTCATCGAGTCTCTCGCCCGTCTCAACCACAGATTGAAGAGCGAGAAGTCCGCCAAGACCAT
TGTGGCCTTTATCATCATGCCCGCCCAGACCTCATCATACACAGTGGAGACCCTCAAGGGCCAGGCCGT
TATGAAGGCTCTGGAGGACACCGTCAACGAAATTCCAGCAGCAGATTGGCCGACGAATGCTCGACCA
TTGTGCCCGCCACAACAGCCACGACGGAAAGGAGGTCCCCGGCCTCGACCAGCTGCTTTCCCCCTTTAG
ATAGTCTTCAGCTCAAGCGACGAGTGTTTGCCCTCAAGCGAGAAACATTCCCCCCCATTGTCACCCACA
ACATGGTGGACGACTCCACAGTCCCCATTCTCAACCAGATCCGACGGGTACAGCTGTTCAACCGGCCC
GAGGACCGGGTCAAGATTATCTTCCACCCCGAGTTCCTCAACTCCAACAACTCTCTCCTGCCTCTCGACT
ACGACGACTTTGTGCGAGGCTGTCATCTCGGAGTCTTCCCCTCCTACTACGAGCCTTGGGGATACACCC
CCGCCGAGTGCACCGTCATGGGCGTTCCTTCCATCACCACCAACCTCTCTGGTTTCGGCTGCTACATGG
AGGATCTCATTGAGAACGCCTCCGACTACGGCATCTACATTGTGGACCGTCGTCTCAAGTCTATTGACG
AGAGTGTAGACCAGTTAACCGACTACATGTTAGATGTCTTCAAGGCACAGCGACGTCAGCGAATTAAC
CCTAGAAAGATAGTCTGCGTAAAATGACGCAGCATTCTTGAAATATGCTCTCTCTTCTAAGTACGATCC