Supplementary Information:

Long-term and Programmable Bacterial Subculture in Completely Automated Microchemostats

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Contents

Supplementary Figures

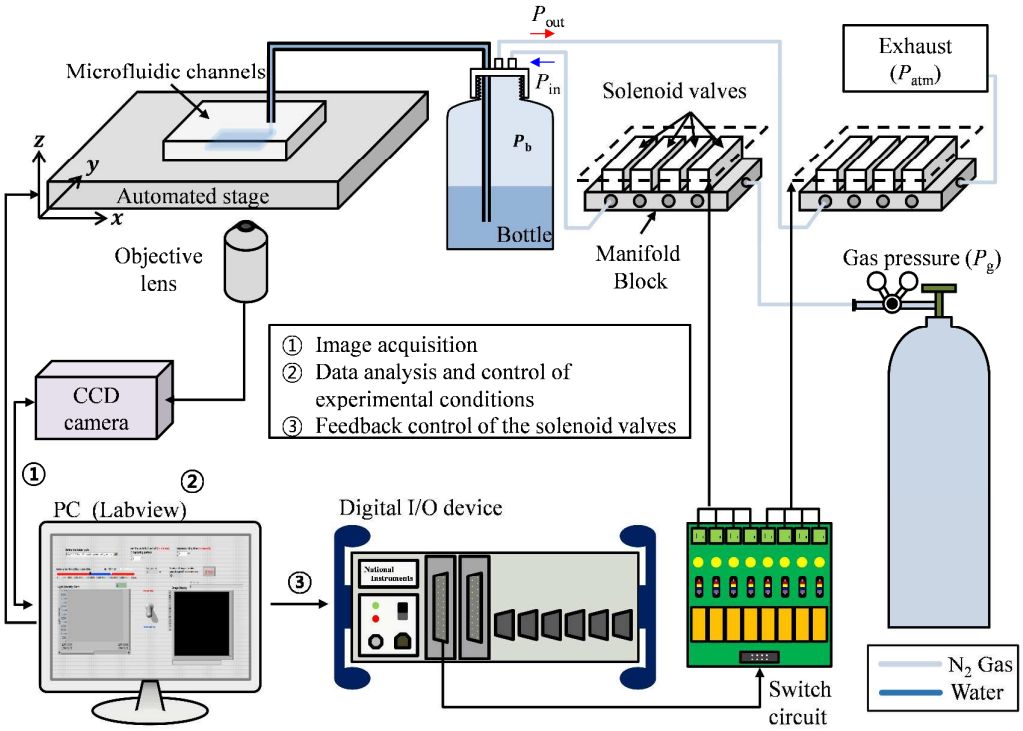


Figure S1. Experimental setup for real-time population measurement and *in situ*, closed-loop, and feedback control of bacterial population. Experimental setup for real-time and closed-loop programming of bacterial subculture. A customized LabVIEW program was used to obtain microscopic images with a CCD camera and then measured their fluorescence intensities in the region of interest. The quantified fluorescence information was used to manipulate solenoid valves for feedback control. During chemostat cultures, the input solenoid valves were normally opened while the output solenoid valves were closed. As a result, the pressure in the bottle (P_b) remained the same as the gas pressure (P_g). Subsequently, the control channel could be pressurized by an incompressible water solution in the bottle, which kept the chemostat chamber closed. For bacterial dilutions, the input solenoid valves were closed while the output solenoid valves were temporally opened to exhaust the compressed gas in the bottle. This resulted in a momentary equilibrium with atmosphere pressure (P_{atm}). The source pressure from the gas tank could be adjusted using a manual regulator.

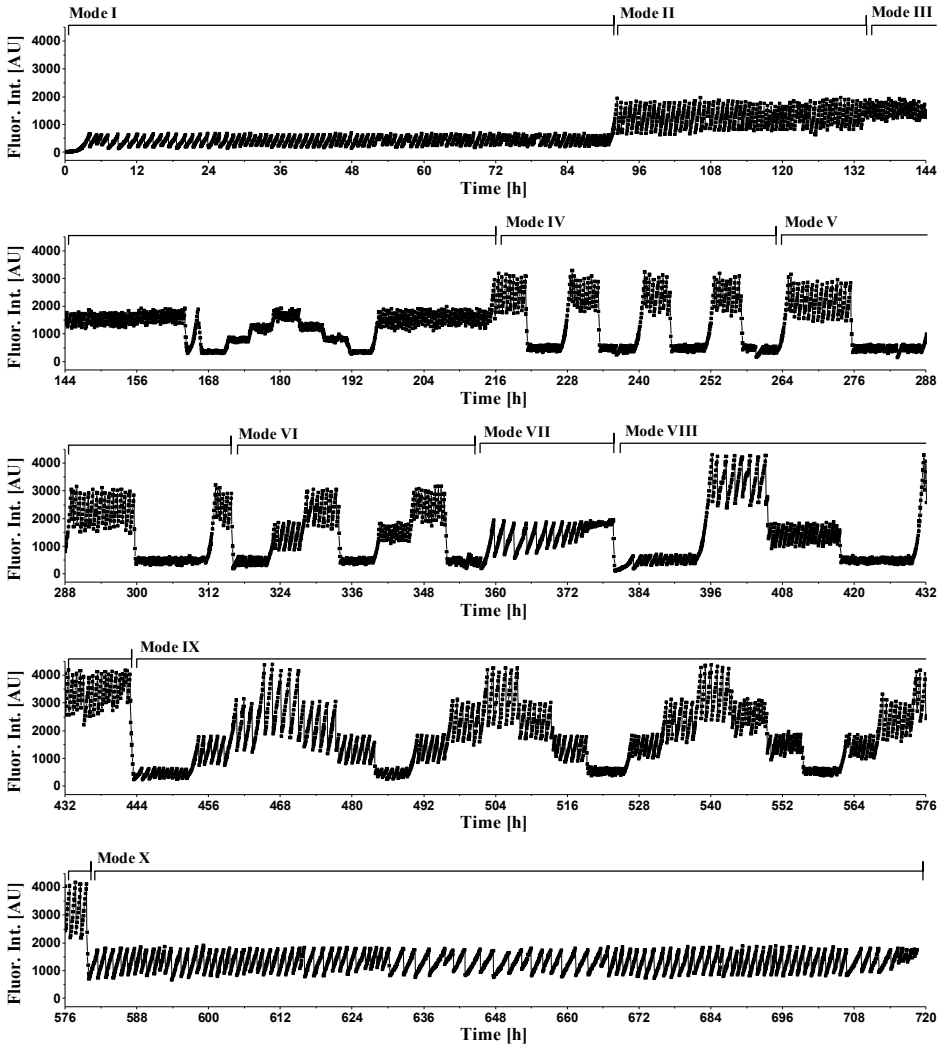
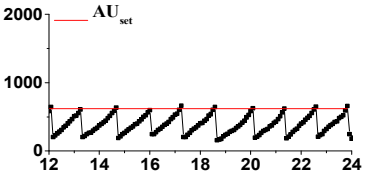
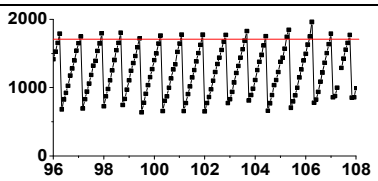
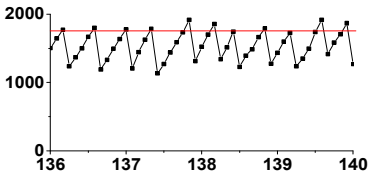
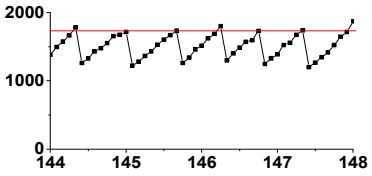
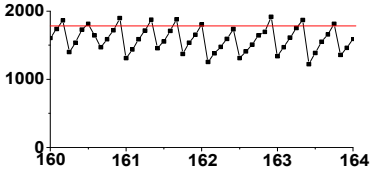
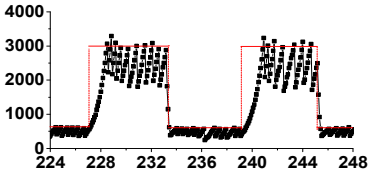
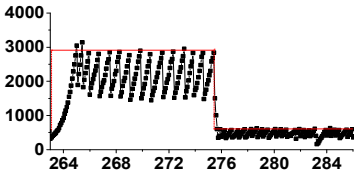
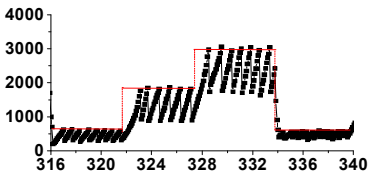
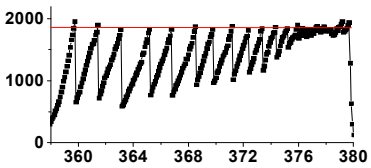
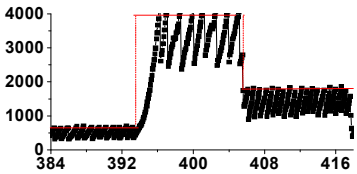
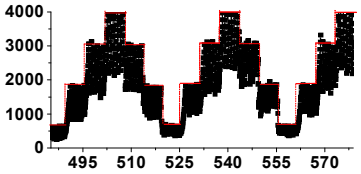


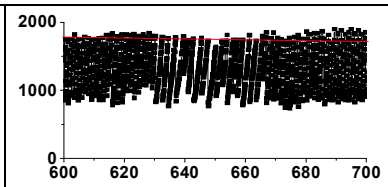
Figure S2. Ultra-long-term bacterial culture. Fully automated and continuous bacterial population growth and subculture programming over 720 h (30 days). During the ultra-long-term cultures, 10 subculture modes were demonstrated ("Mode I" through "Mode X").

Supplementary Table

Table S1. Ten representative subculture modes, experimental conditions used, and analysis results are summarized from the full growth behaviour and subculture programming data in Figure S2.

Mode (period)	**Subculture results**	**Experimental conditions and analysis results**	**Remarks and potential applications**
I (0–90 h)		▪ FI$_{set}$ = 600 ▪ t_{open} = 2.5 s, $t_{capture}$ = 5 min, Q = 25 µL/h ▪ CC5[†] = 1× TB with 1× ampicillin ▪ FI$_{max}$ = 628 ± 12, FI$_{min}$ = 228 ± 22 ▪ Δt = 0.84 ± 0.11 [h], ▪ μ = 1.21 [/h] ▪ m = 476 ± 41 [AU/h]	- 38.9% volume fraction (38.9%) - Potential applications in analysis of cellular growth kinetics[1]
II (90–133 h)		▪ FI$_{set}$ = 1700 ▪ t_{open} = 2.5 s, $t_{capture}$ = 5 min, Q = 25 µL/h ▪ CC = 1× TB with 1× ampicillin ▪ FI$_{max}$ = 1795 ± 36, FI$_{min}$ = 801 ± 27 ▪ Δt = 0.66 ± 0.06 [h], ▪ μ = 1.22 [/h] ▪ m = 1506 ± 99 [AU/h]	- Increased FI$_{set}$ value - Conservation of the volume fraction rate (40.7%)
III-1 (133–143 h)		▪ FI$_{set}$ = 1700 ▪ t_{open} = 1.5 s, $t_{capture}$ = 5 min, Q = 25 µL/h ▪ CC = 1× TB with 1× ampicillin ▪ FI$_{max}$ = 1805 ± 32, FI$_{min}$ = 1277 ± 36 ▪ Δt = 0.29 ± 0.03 [h], ▪ μ = 1.19 [/h] ▪ m = 1820 ± 124 [AU/h]	- ½ chamber opening time - 1× antibiotic dose
III-2 (143–153 h)		▪ t_{open} = 1.5 s, $t_{capture}$ = 5 min, Q = 25 µL/h ▪ CC = 1× TB with 2× ampicillin ▪ FI$_{max}$ = 1751 ± 23, FI$_{min}$ = 1290 ± 25 ▪ Δt = 0.44 ± 0.05 [h], ▪ μ = 0.69 [/h] ▪ m = 1047 ± 68 [AU/h]	- 2× antibiotic dose - Potential applications in antibiotic susceptibility tests[2]
III-3 (153–164 h)		▪ t_{open} = 1.5 s, $t_{capture}$ = 5 min, Q = 25 µL/h ▪ CC = 1× TB with 2× ampicillin ▪ FI$_{max}$ = 1830 ± 23, AU$_{min}$ = 1334 ± 26 ▪ Δt = 0.31 ± 0.02 [h], ▪ μ = 1.02 [/h] ▪ m = 1600 ± 175 [AU/h]	- Adaptation to 2× antibiotic dose - Potential applications in evolutionary adaptation[3]
IV (195–263 h)		▪ FI$_{set}$ = 550 (6 h) → 3000 (6 h) ▪ t_{open} = 1.5 s, $t_{capture}$ = 5 min, Q = 25 µL/h ▪ CC = 1× TB with 2× ampicillin ▪ FI$_{max}$ = 587 ± 11 → 3061 ± 49 ▪ FI$_{min}$ = 365 ± 26 → 1958 ± 77 ▪ Δt = 0.44 ± 0.08 [h] → 0.43 ± 0.07 [h] ▪ μ = 1.08 [/h] → 1.04 [/h] ▪ m = 504 ± 21 → 2565 ± 345 [AU/h]	- μ_{AU} = 1.10 recovery - Programming two FI$_{set}$ values - Potential applications in programmed bioprocess and biotechnology[4]

V (263–316 h)		▪ FI_{set} = 3000 (12 h) → 550 (12 h) ▪ t_{open} = 1.5 s, $t_{capture}$ = 5 min, Q = 25 µL/h ▪ CC = 1× TB with 2× ampicillin ▪ FI_{max} = 3074 ± 50 → 583 ± 6 ▪ FI_{min} = 1754 ± 96 → 362 ± 16 ▪ Δt = 0.51 ± 0.07 [h] → 0.40 ± 0.02 [h] ▪ μ = 1.10 → 1.19 [/h] ▪ m = 2588 ± 300 → 542 ± 23 [AU/h]	- Two-step programming with a 2×-long period
VI (316–358 h)		▪ FI_{set} = 550 (6 h) → 1700 (6h) → 3000 (6 h) ▪ t_{open} = 1.5 s, $t_{capture}$ = 5 min, Q = 25 µL/h ▪ CC = 1× TB with 2× ampicillin ▪ FI_{max} = 574 ± 22 → 1783 ± 25 → 3021 ± 45 ▪ FI_{min} = 345 ± 22 → 1147 ± 28 → 1919 ± 74 ▪ Δt = 0.43 ± 0.05 → 0.41 ± 0.10 → 0.44 ± 0.05 [h] ▪ μ = 1.18 → 1.08 → 1.03 [/h] ▪ m = 542 ± 77 → 1699 ± 182 → 2539 ± 179 [AU/h]	- Three-step programming
VII (358–380 h)		▪ FI_{set} = 1700 ▪ t_{open} = 1.5 s, $t_{capture}$ = 5 min, Q = 5–25 µL/h ▪ CC = 1× TB with 2× ampicillin ▪ FI_{max} = 1855 ± 22 ▪ FI_{min} = 1153 ± 429 ▪ Δt = [h] = 1.04 ± 0.54 ▪ μ = 0.92 [/h] ▪ m = 762 ± 65 [AU/h]	- Flow rate programming
VIII (380–444 h)		▪ FI_{set} = 550 (12 h) → 4000 (12 h) →1700 (12 h) ▪ t_{open} = 1.5 s, $t_{capture}$ = 5 min, Q = 25 µL/h ▪ CC = 1× TB with 2× ampicillin ▪ FI_{max} = 614 ± 23 → 4102 ± 71 → 1778 ± 30 ▪ FI_{min} = 375 ± 26 → 2653 ± 128 → 1067 ± 41 ▪ Δt = 0.50 ± 0.17 → 0.50 ± 0.11 → 0.54 ± 0.04 [h] ▪ μ = 0.99 → 0.87 → 0.95 [/h] ▪ m = 528 ± 50 → 2261 ± 323 → 1299 ± 54 [AU/h]	- Three-step and nongradual programming
IX (444–580 h)		▪ FI_{set} = 550 (6h) → 1700 (6 h) → 3000 (6 h) → 4000 (6h) → 3000 (6h) → 1700 (6 h) → 550 (6 h) ▪ t_{open} = 1.5 s, $t_{capture}$ = 5 min, Q = 25 µL/h ▪ CC = 1× TB with 2× ampicillin ▪ FI_{max} = 650 ± 27 → 1824 ± 38 → 3050 ± 61 → 4308 ± 46 → 3039 ± 62 → 1843 ± 52 → 635 ± 18 ▪ FI_{min} = 423 ± 31 → 1060 ± 59 → 1649 ± 60 → 2046 ± 43 → 1727 ± 195 → 1087 ± 115 → 434 ± 34 ▪ Δt = 0.38 ± 0.09 → 0.55 ± 0.08 → 0.64 ± 0.09 → 0.76 ± 0.14 → 0.62 ± 0.11 → 0.57 ± 0.04 → 0.40 ± 0.07 [h] ▪ μ = 1.13 → 0.99 → 0.96 → 0.98 → 0.91 → 0.93 → 0.95 [/h] ▪ m = 597 ± 39 → 1389 ± 123 → 2189 ± 44 → 2976 ± 122 → 2116 ± 129 → 1326 ± 31 → 502 ± 15 [AU/h]	- Multistep programming

X (580–720h)		▪ FI_{set} = 1800 ▪ t_{open} = 1.5 s, $t_{capture}$ = 5 min, Q = 25 μL/h ▪ CC = 1–0.2× TB with 2× ampicillin ▪ FI_{max} = 1758.7 ± 61 ▪ FI_{min} = 915 ± 88 ▪ Δt = 0.87 ± 0.36 ▪ μ = 0.94 (1×) → 0.95 (0.8×) → 0.89 (0.6×) → 0.81 (0.4×) → 0.66 (0.2×) → 0.62 (0.4×) → 0.86 (0.6×) → 0.98 (0.8×) → 0.95 (1.0×) [/h] ▪ m = 1100 (1×) → 1133 (0.8×) → 1057 (0.6×) → 902 (0.4×) → 696 (0.2×) → 629 (0.4×) → 967 (0.6×) → 1166 (0.8×) → 1211 (1×) [AU/h]	- Grow factor concentration programming - Potential applications in nutrient optimization[5 6]

[†]CC: culture condition.
Note that the culture temperature was fixed at 35°C unless otherwise indicated.
All the *x*- and *y*-axes of the graphs from I through X indicate the time in hours and the fluorescent intensity in arbitrary units (AU), respectively.

References

(1) Kovarova-Kovar, K; Egli, T. *Microbiol. Mol. Biol. R.* **1998**, 62, 646-666.

(2) Barczak, A. K.; Gomez, J. E.; Kaufmann, B. B.; Hinson, E. E., Cosimi, L.; Borowsky, M. L.; Onderdonk, A. B.; Stanley, S. A.; Kaur, D.; Bryant, K. F.; Knipe, D. M.; Sloutsky, A.; Hung, D. T. *Proc. Nat. Acad. Sci. USA.* **2012**, 109, 6217-6222.

(3) Toft, C; Andersson, S. G. E. *Nat. Rev. Genet.* **2010**, 11, 465-475.

(4) Linko, P.; Zhu, Y. H. *J. Biotechnol.* **1991**, 21, 253-269.

(5) Sirisantimethako, L.; Sanchanda, P.; Chatleudmongkol, J.; Laopaiboon, L.; Laopaiboon, P. *Curr. Opin. Biotech.* **2013**, 24, 137-137.

(6) Li, Y. Q.; Jiang, H. X.; Xu, Y. Q.; Zhang, X. H. *Appl. Microbiol. Biot.* **2008**, 77, 1207-1217.