Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Arrayed labeling-free cultivation and growth evaluation from a single microorganism

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Reagent.

Table S1. The utilized reagents and solvents. All reagents and solvents employed for this study were used as supplied (Table S1). The pure water was supplied by a Milli-Q water purification system (18 MΩ·cm at 25 °C).

Reagents	**CAS No.**	**[a] Supplier, [b] code, and [c] grade**
Sulforhodamine B	3520-42-1	[a] FUJIFILM Wako Pure Chemical Corporation, [b] 152479
SYTO9	-	[a] Thermo Fisher Scientific, [b] S34854
Yeast extract	8013-01-2	[a] Thermo Fisher Scientific, [b] 211929
Peptone	73049-73-7	[a] Thermo Fisher Scientific, [b] 211677
Sodium chloride	7647-14-5	[a] FUJIFILM Wako Pure Chemical Corporation, [b] 191-01665, [c] 99.5+%
D-glucose	50-99-7	[a] FUJIFILM Wako Pure Chemical Corporation, [b] 049-31165, [c] 98.0+%
Photoresist	-	[a] Zeon Corporation, [b] ZPN1150
Polydimethylsiloxane	9016-00-6	[a] FUJIFILM Wako Pure Chemical Corporation, [b] 04019862
Carbonated water	-	[a] Asahi Holdings, Inc., [b] 2T251
Cytop (amorphous perfluoropolymer)	-	[a] AGC Inc., [b] CTX-809SP2
Sodium hydroxide	1310-73-2	[a] FUJIFILM Wako Pure Chemical Corporation, [b] 191-01665, [c] 93.0+%
Hydrochloric acid	7647-01-0	[a] FUJIFILM Wako Pure Chemical Corporation, [b] 087-01076, [c] 35.0–37.0%

Table S2. Experimental instruments. The standard photolithography process was performed in a yellow clean room (class 100) at the AIST Nano-Processing Facility (NPF), Tsukuba, Japan.

Instruments	**[a] Brands and [b] types**	**Conditions**
Spincoater	[a] Mikasa Co., Ltd., [b] 1H-07	-
pH meter	[a] HORIBA, Ltd., [b] D-71 LAQUAact Handheld Meter	-
Compact pH meter	[a] HORIBA, Ltd., [b] LAQUAtwin pH-22B	-
Laser microscope	[a] Olympus Corporation, [b] LEXT OLS4100	10×magnification
Fluorescence microscope	[a] Olympus Corporation, [b] IX71	40×magnification
Incubator	[a] Taitec Corporation, [b] BioShaker BR-13FP	Temperature 37°C, 180 rpm
Digital hot plate	[a] As One Corporation, [b] DP-2S	-
UV-vis spectrophotometer	[a] Hitachi, Ltd., [b] U-5100	-
Maskless exposure system	[a] NanoSystem Solutions, Inc., [b] DL1000	-

Preparation of a picoliter-sized incubator array

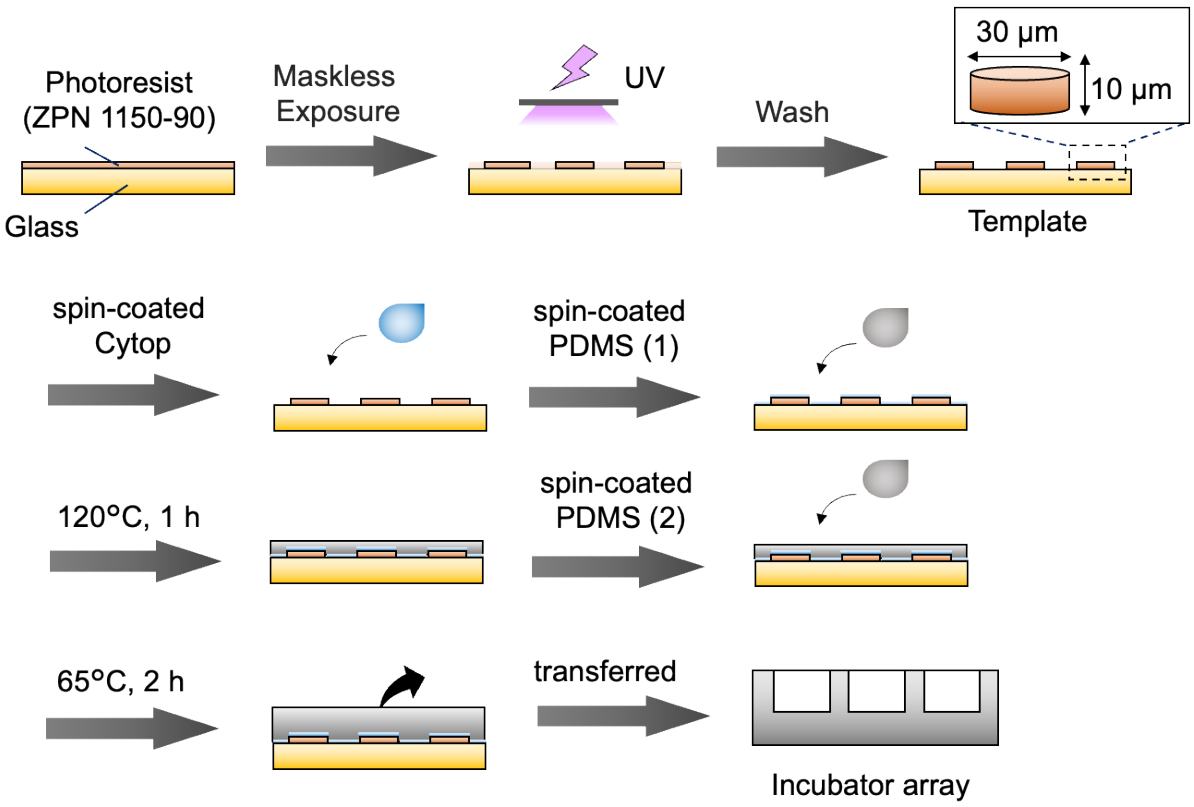

Fig. S1. Fabrication scheme of the picoliter-sized incubator array. The incubator pattern was designed by 2D CAD software (DraftSight, Dassault Systèmes S.E., France). The designed diameter of the incubator was 30 μm. A photoresist (ZPN 1150-90, Zeon Corp., Japan) was first spin-coated on a glass substrate at a rotation speed of 2500 rpm for 30 s. Then, the photoresist film was patterned with a standard photolithographic process. The patterned residues of the photoresist film (micropillars with a height of about 10 μm) were utilized as a template for the incubator array. Next, an amorphous fluoropolymer (Cytop CTX-809SP2, AGC Inc., Japan) in a fluorinert solvent (CT-solv.180, AGC Inc., Japan) was deposited on the template with a spin-coating technique (rotation speed: 4000 rpm). After that, a PDMS film was deposited on the fluoropolymer-coated substrate. The thin-film structure contributes to the suppression of the self-bending phenomenon exhibited by substrates because of their internal stress. This means that interfacial adhesion between the PDMS incubator array and a glass dish can be maintained by employing the thin-film structure. In this regard, we employed the spin-coating deposition process for preparing the PDMS-based incubator array. An oligomer solution of PDMS (Sylgard 184, Dow Chemical Company, USA) with a curing agent was spin-coated and cured on the template. The final thickness of the PDMS film was roughly 20 μm. Then, the completed PDMS film was peeled from the template. The pattern of the PDMS film was confirmed using a LEXT OLS4100 laser scanning microscope (Olympus, Japan).

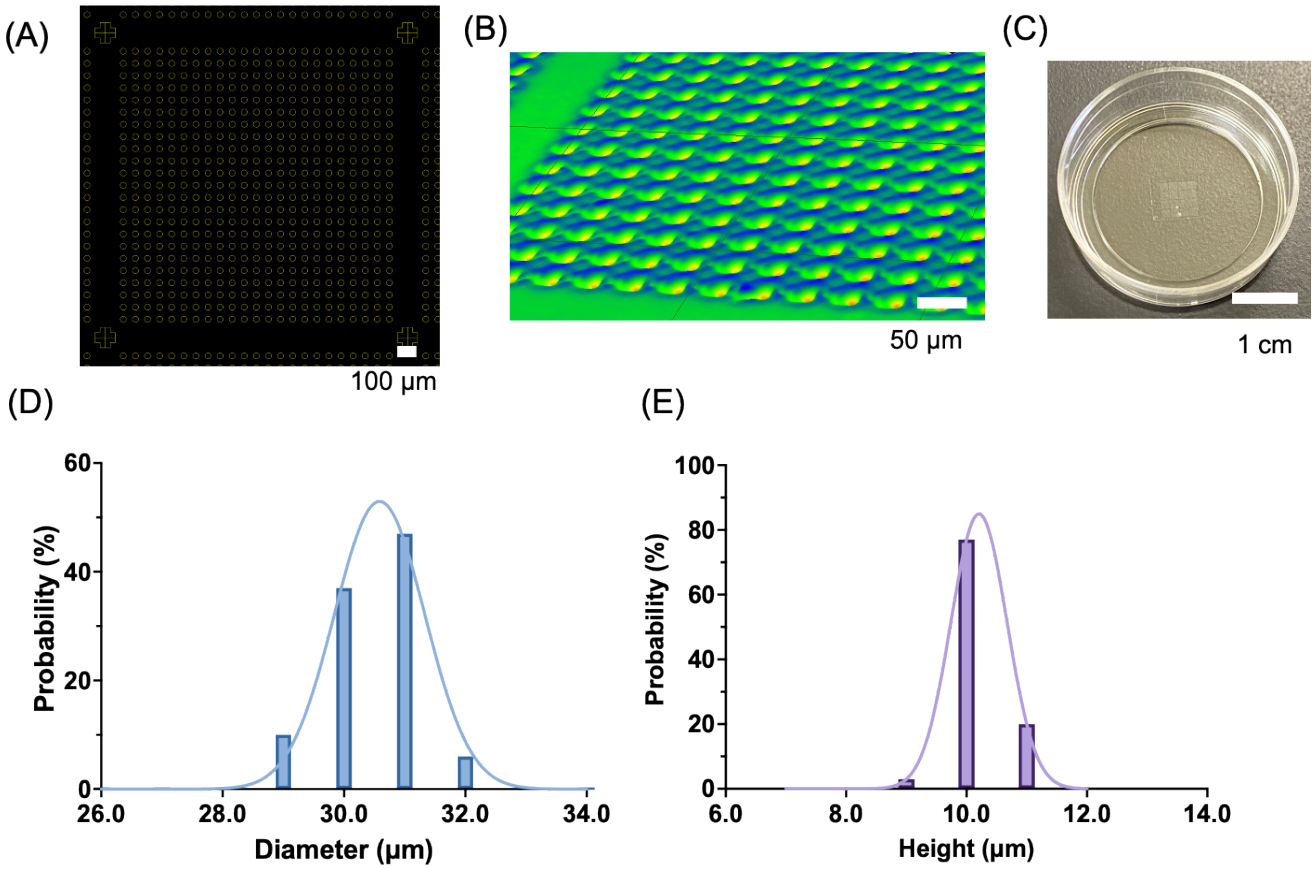

Fig. S2. (A) Picoliter-sized incubator array designed with CAD software. The pitch of each incubator was designed to be 30 μm. (B) Laser microscopic image of the fabricated 92 × 92 incubator array. (C) Photograph of the incubator array attached to a glass-bottom dish. (D, E) Measurement of diameter or height of picoliter-sized incubator. Average diameter and height of each incubator was 30.46 ± 0.68 μm and 10.28 ± 0.37 μm, respectively. Data points were fitted to the sum of the Gaussian distributions (N=100).

Microorganism compartmentalization

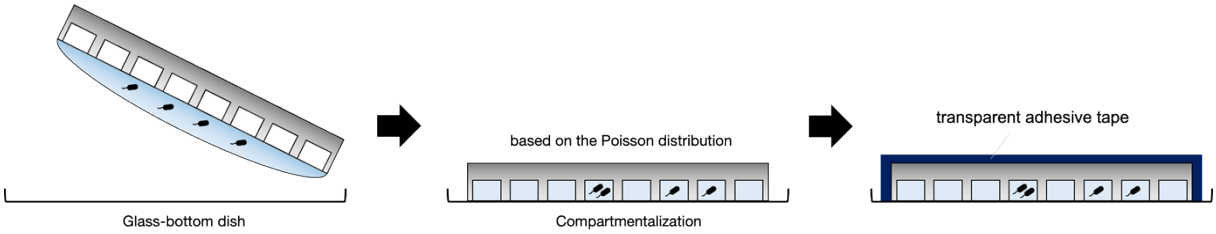

Fig. S3. A schematic of the compartmentalization of single *E. coli* into each picoliter-sized incubator array.

Compartment and leakage test of small molecule

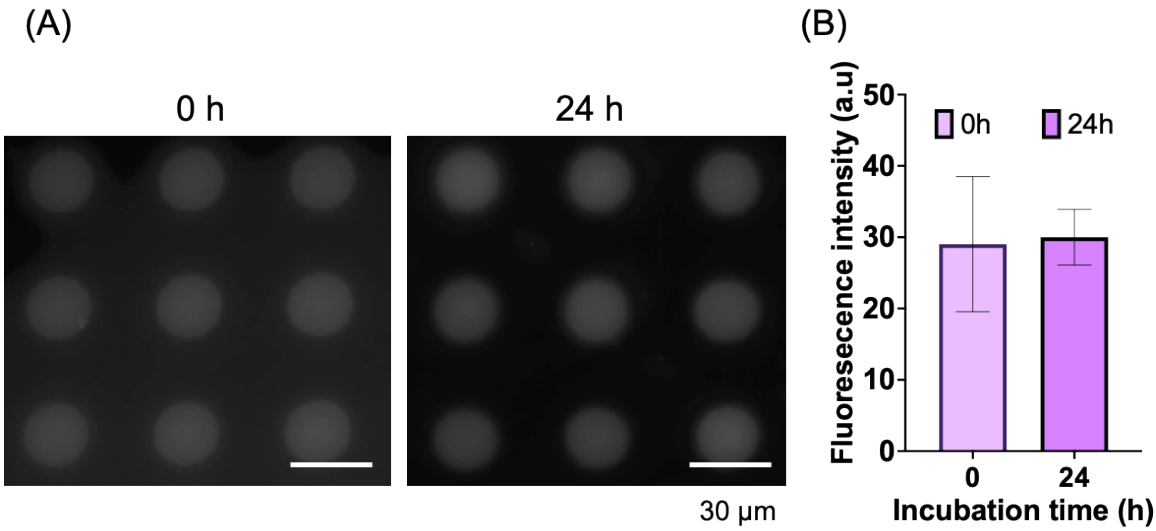

Fig. S4. Leakage test of enclosed fluorescence indicator (1000 μM sulforhodamine B). (A) Fluorescence images under a microscope before and after 24 h incubation. (B) Fluorescence intensities of the images calculated with ImageJ (N=20). Error bars indicate the standard deviation.

Cultivation of *E. coli*.

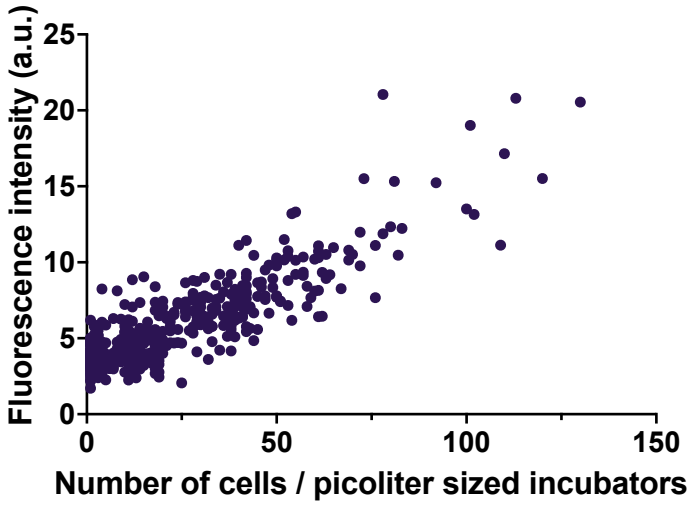

Fig. S5. The collection of fluorescence intensity and number of cells in picoliter sized incubators. The *E. coli* was cultured on our incubator array, and the growth was evaluated from time-lapse autofluorescence intensity of *E. coli*. R=0.8593.

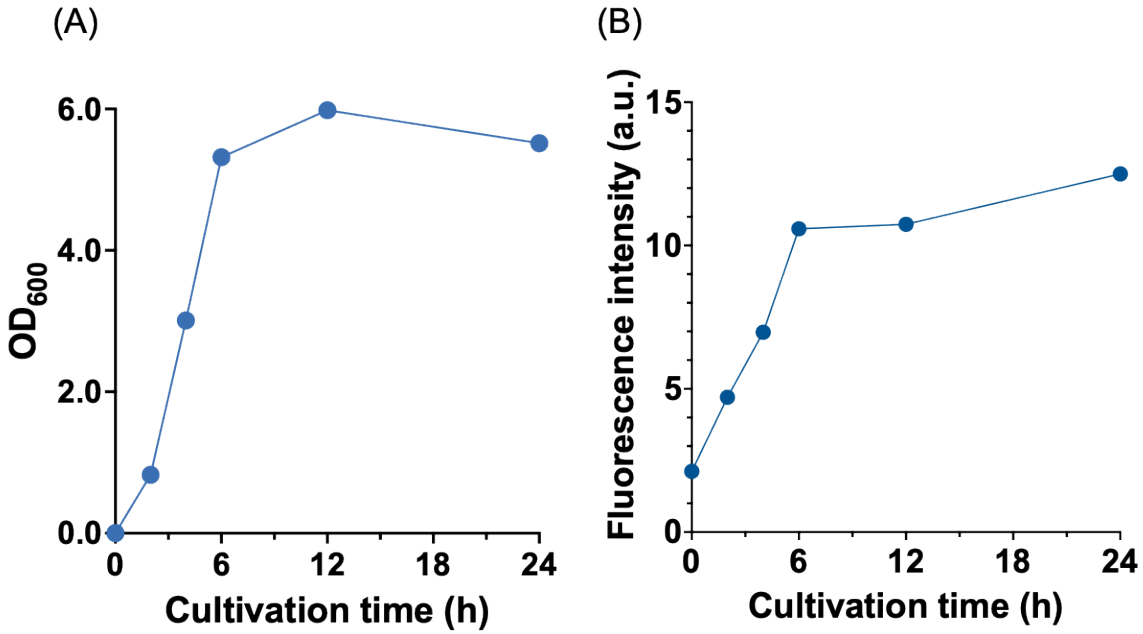

Fig. S6. The growth curves of *E. coli* K-12 in (A) conventional and (B) picoliter sized incubators. (A); *E. coli* was cultured in a flask with 5 mL of medium. The growth was evaluated from OD_{600} with a UV-vis spectrophotometer, after the medium had been collected at each cultivation time. (B); *E. coli* was cultured on our incubator array, and the growth was evaluated from time-lapse autofluorescence intensity of *E. coli*.

Table S3. The maximum growth rate of *E. coli* in conventional and picoliter sized incubators. The maximum growth rates in the flask and picoliter sized incubators were calculated by analyzing the change of optical density or the autofluorescence intensity. The maximum growth rate was determined by fitting the experimental data to the growth curve using Combase database (www.combase.cc).

	Minimum growth rate (1/h)
conventional method	1.273 ± 0.127
picoliter sized incubators	1.399 ± 0.213

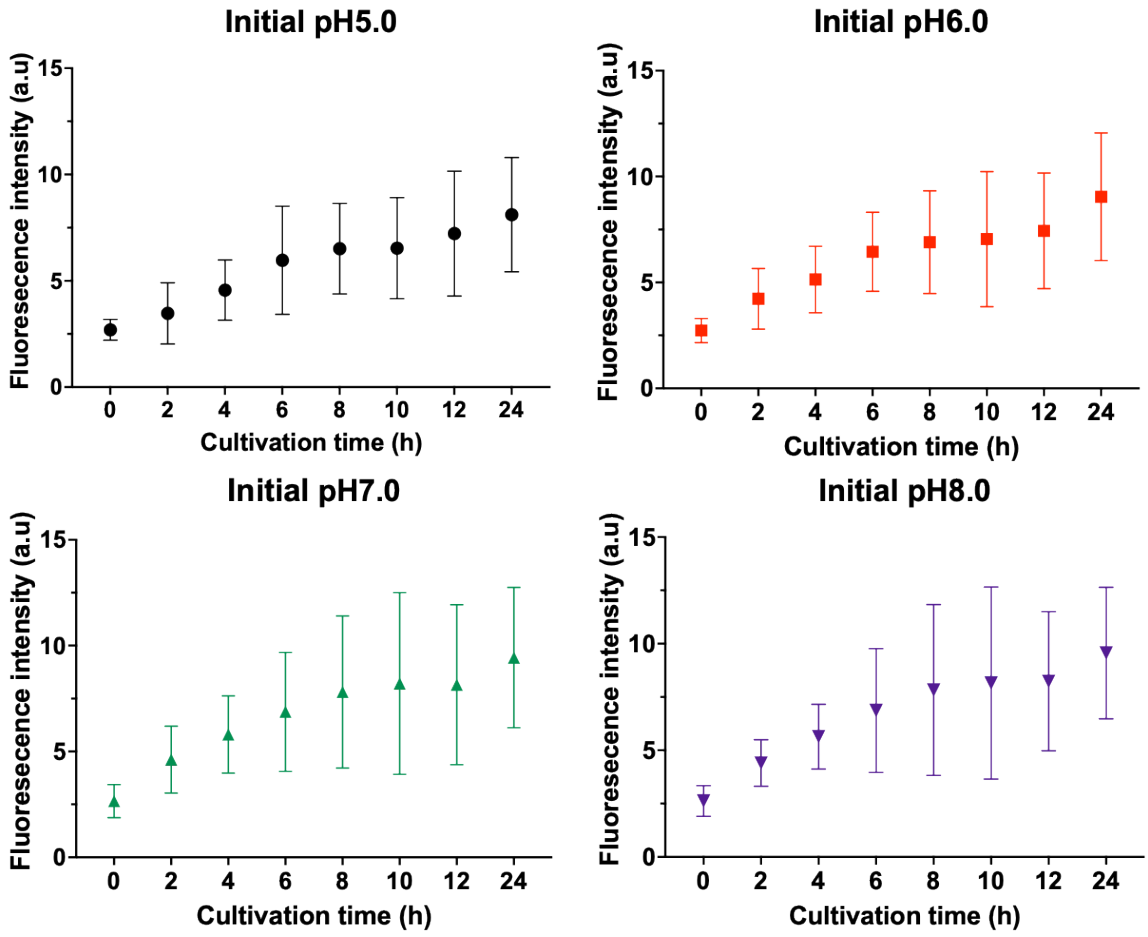

Fig. S7. The mean and standard deviation of the autofluorescence intensity on the incubator array. 100 single *E. coli* were compartmentalized into individual incubators and cultured for 24 h with different pH values in the initial state. Each plot and line indicate mean or standard deviation. N=100.

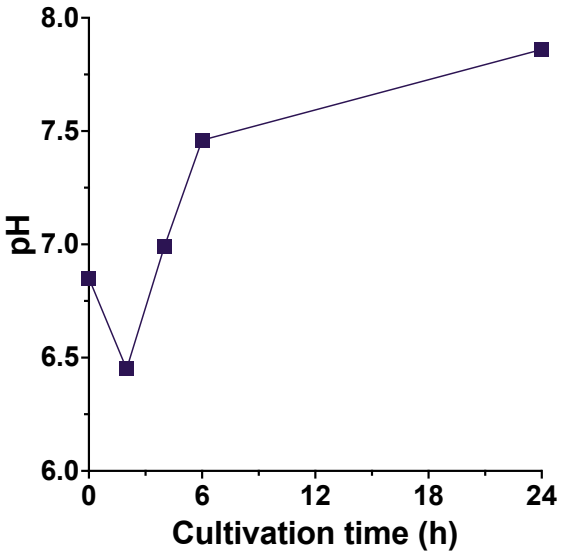

Fig. S8. The changes of pH in the flask cultivation. *E. coli* was cultured in a flask with 5 mL of medium. The change of pH was measured by compact pH meter (LAQUAtwin, HORIBA's, Japan).

Table S4. The minimum and maximum values of the autofluorescence intensity on the incubator array after 24 h when varying the initial pH of the medium. The data were selected from among 100 *E. coli*.

	Minimum	Maximum
IpH5.0	4.20	16.76
IpH6.0	4.55	22.35
IpH7.0	4.31	25.15
IpH8.0	5.12	22.68

Table S5. The mean and standard deviation of the autofluorescence intensity on the incubator array when varying cultivation time and initial pH of medium.

	0 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h
IpH5.0	2.70 ± 0.49	3.47 ± 1.43	4.56 ± 1.41	5.97 ± 2.53	6.51 ± 2.12	6.53 ± 2.37	7.22 ± 2.92	8.11 ± 2.67
IpH6.0	2.81 ± 0.71	4.15 ± 1.42	5.03 ± 1.56	6.16 ± 1.86	6.37 ± 2.41	6.37 ± 3.17	6.68 ± 2.71	8.54 ± 3.00
IpH7.0	2.65 ± 0.78	4.61 ± 1.57	5.80 ± 1.81	6.87 ± 2.80	7.81 ± 3.58	8.21 ± 4.27	8.15 ± 3.76	9.43 ± 3.30
IpH8.0	2.62 ± 0.71	4.40 ± 1.09	5.64 ± 1.51	6.87 ± 2.89	7.83 ± 3.98	8.16 ± 4.48	8.24 ± 3.24	9.56 ± 3.07

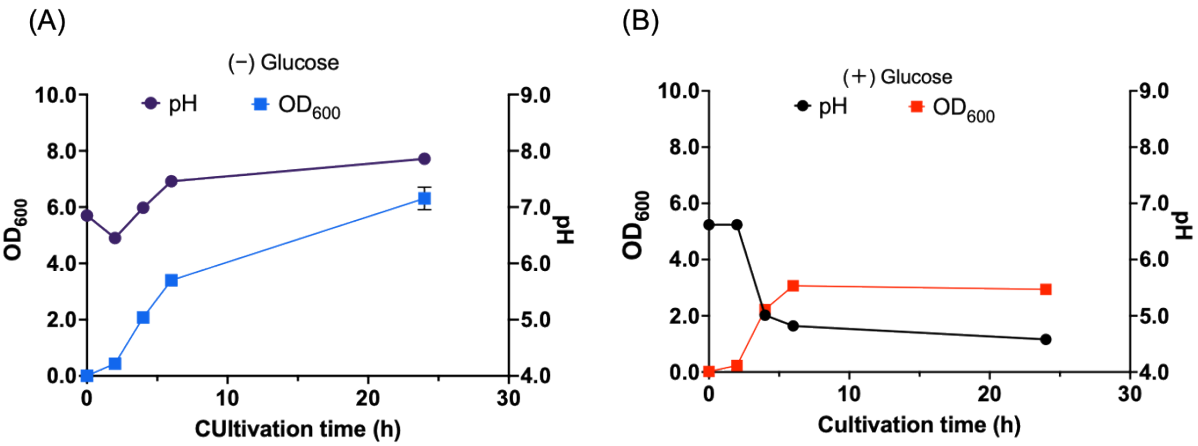

Fig. S9. Evaluation of *E. coli* growth in the presence or absence of D-glucose (5%) in a conventional flask. Blue and red lines indicate the growth curve of *E. coli*, and black line indicates pH in LB medium.

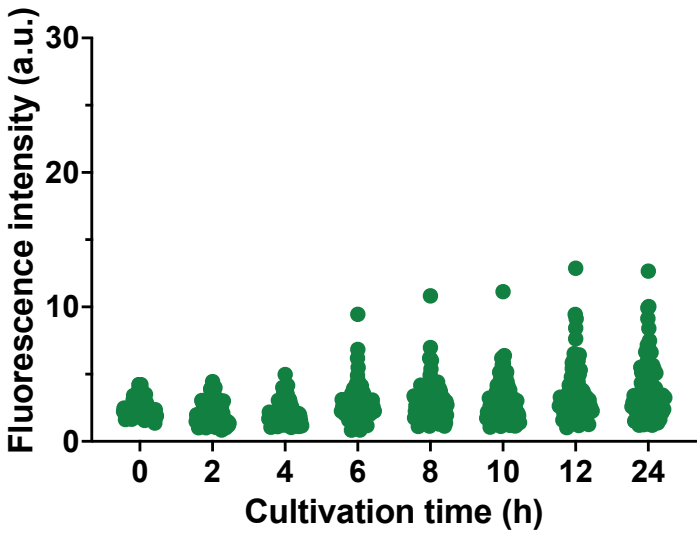

Fig. S10. Evaluation of *E. coli* growth in the presence of D-glucose (5%).

Table S6. The mean and standard deviation of the autofluorescence intensity on the incubator array when the *E. coli* was cultivated in the medium including 5% D-glucose.

	0 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h
5% Glucose	2.47 ± 0.61	2.09 ± 0.80	2.16 ± 0.77	2.67 ± 1.24	2.95 ± 1.40	2.96 ± 1.50	3.49 ± 1.99	3.79 ± 2.12