Supplementary Material (ESI) for Lab on a Chip


# SUPPLEMENTARY INFORMATION for Isolation, incubation, and parallel functional testing and identification by FISH of rare microbial single-copy cells from multi-species mixtures using the combination of chemistrode and stochastic confinement

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## Supplementary Methods

### Materials

Unless otherwise stated, all chemicals were purchased at standard grades and used as received. FC40 (3M) was used as the carrier fluid with the surfactant RfOEG (triethyleneglycolmono[1*H*,1*H*-perfluorooctyl]ether), which was prepared according to published procedures.[1]

Soil used for Fig.1b was collected from haphazardly chosen locations in pots containing houseplants that are kept in the atrium of the Gordon Center for Integrative Sciences at the University of Chicago. The soil was crushed with a mortar and then sieved by using a 38 μm test sieve (Fisherbrand). The soil slurry was made by suspending the soil in DI water with a concentration of soil of approximately 400 g/L.

### Fabrication and operation of microfluidic devices

Microfluidic devices were fabricated by using soft lithography[2] as described previously.[1] Flows in the microfluidic devices were controlled as described previously.[3]

### Acquisition and analysis of microscopic images

Images in Figs. 1a, 3a, 3b, 4b, 4c, and 6e were taken by using a stereoscope (MZ $12_5$, Leica) equipped with a camera (SPOT insite QE, Diagnostic Instruments Inc.). The images in Figs. 1b, 2, 3c, 4a and 4d were taken by using an epi-fluorescence microscope (DMI6000, Leica) equipped with a filter set of an L5 (Leica) and a TX2 (Leica) and a 20× objective (0.40 NA). Fluorescent images were taken at 200 ms exposure time and processed with appropriate background scales using MetaMorph image software (Molecular Devices). Fluorescent images taken with the L5 filter were processed with a low scale value (L) of 250 and a high scale value (H) of 270 for Fig. 2b, L260 H280 for Fig. 2c, and L290 H400 for Fig. 4d. Fluorescent images taken with the TX2 filter were processed with a low scale value (L) of 200 and a high scale value (H) of 4095 for Fig. 2b, L190 H3300 for Fig. 2c, L390 H550 for Fig. 4a, and L250 H350 for Fig. 4d. Each fluorescent image in Fig. 2 and Fig. 4d was overlaid by two images taken with L5 and TX2 filters.

**Supplementary Figures and Table**

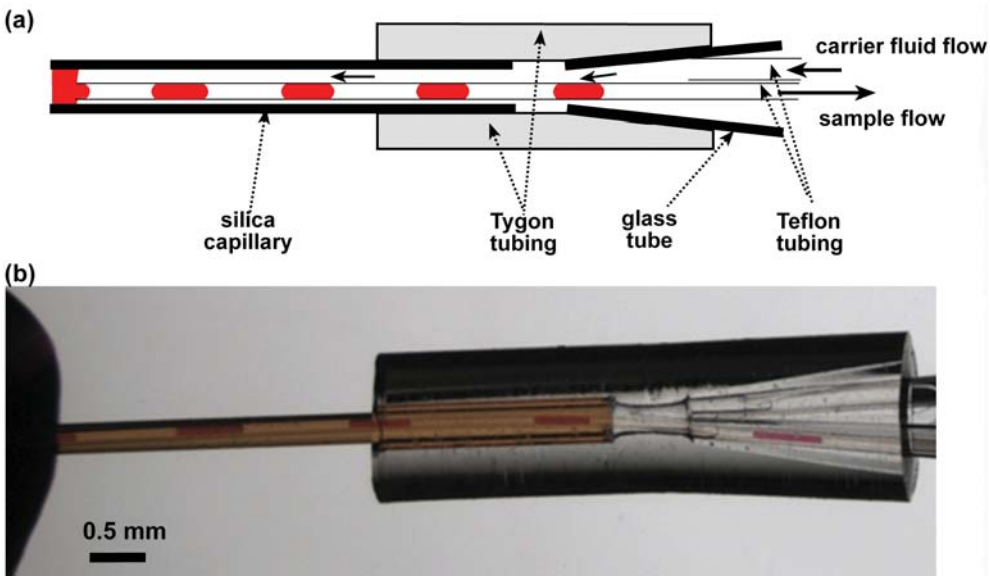


**Fig. S1.** The structure of the chemistrode modified for sampling directly from the environment. a) A schematic of the design of the sampling probe. See Experimental section in main text for detailed description and procedures. b) A photograph shows the connective junction of the modified chemisotrde, by which the plugs were sampled form a solution of red food dye (August Thomsen Corp) and transported through the tubing.

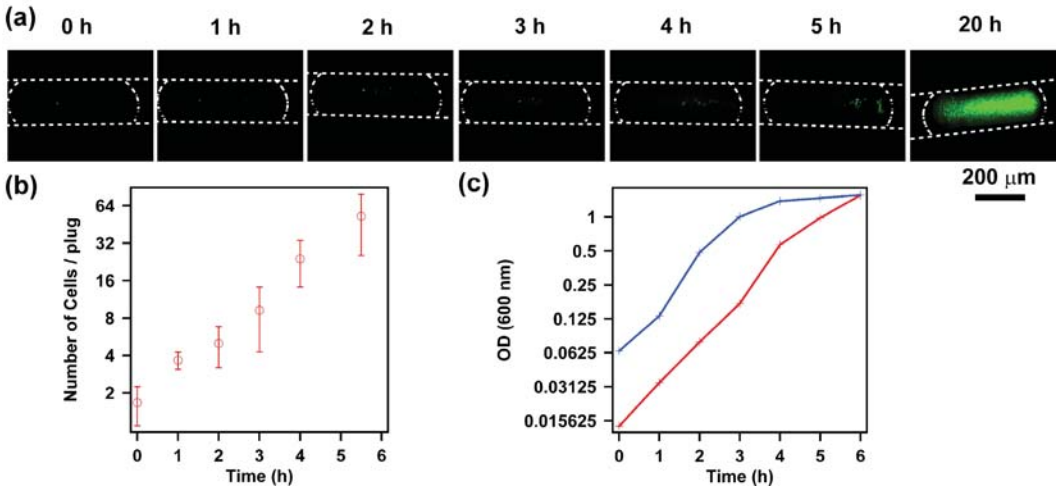


**Fig. S2.** Cells that were stochastically isolated in individual plugs divide and form populations in the plugs, and the dynamics of growth in plugs are similar to those in bulk solution. (a) Stochastically isolated cells of GFP-labeled *E. coli*, isolated in plugs containing TSB and incubated at 37ºC, divided to form populations. A series of time-lapse false color images, taken by using an epi-fluorescence microscope (DMI6000, Leica) equipped with a L5 (Leica) filter set and a 20× objective (0.40 NA), shows the growth in a plug of stochastically isolated cells of GFP-labeled *E. coli* into a population of up to thousands of cells. (b) The growth curve for the early exponential phase of cells in plugs, measured by counting the number of cells per single plug, is shown. Cell counts may be lower than actual numbers of viable cells because clusters of cells could not be distinguished from single cells. Error bars denote standard deviations (n=4). (c) The growth rate of GFP-labeled *E. coli* during the exponential phase in bulk was close to that in plugs (generation time: ~ 60 min in plugs, ~ 50 min in bulk). Cells were grown in bulk solution of TSB media at 37ºC, and the cell density was measured as optical density (O.D.) by using a UV/Vis spectrophotometer (Agilent). The blue trace and the red trace represent two trials of growth of GFP-labeled *E. coli* in bulk from different initial cell densities.

**Table S1.** Detailed data for Fig. 3e.

| Number of plugs | Ratio of *E. coli*:*P. curdlanolyticus* | Estimated input number of cells of *E. coli*[a] | Expected recovered number of populations of *E. coli*[a] | Actual recovered number of populations of *E. coli* | Estimated input number of cells of *P. curdlanolyticus*[a] | Expected recovered number of populations of *P. curdlanolyticus*[b, c] | Actual recovered number of populations of *P. curdlanolyticus* |
|---|---|---|---|---|---|---|---|
| 151 | 1.2:1 | 157 | 98 | 113 | 127 | 30 | 9 |
| 421 | 2:1 | 104 | 92 | 85 | 56 | 41 | 40 |
| 282 | 2.5:1 | 44 | 40 | 50 | 17 | 15 | 17 |
| 501 | 3.5:1 | 115 | 103 | 67 | 34 | 26 | 9 |
| 940 | 15:1 | 165 | 152 | 222 | 11 | 9 | 2 |
| 1052 | 40:1 | 354 | 301 | 254 | 8 | 5 | 3 |

a. Estimated input number of cells for each species was determined by culturing aliquots of pure suspension of that species on agar plates.

b. Expected recovered numbers of populations were calculated by Poisson distribution.

c. Assuming that if both a *P. curdlanolyticus* cell and an *E. coli* cell are isolated together in one plug, *E. coli* would dominate the growth in that plug and the *P. curdlanolyticus* in that plug could not be recovered.

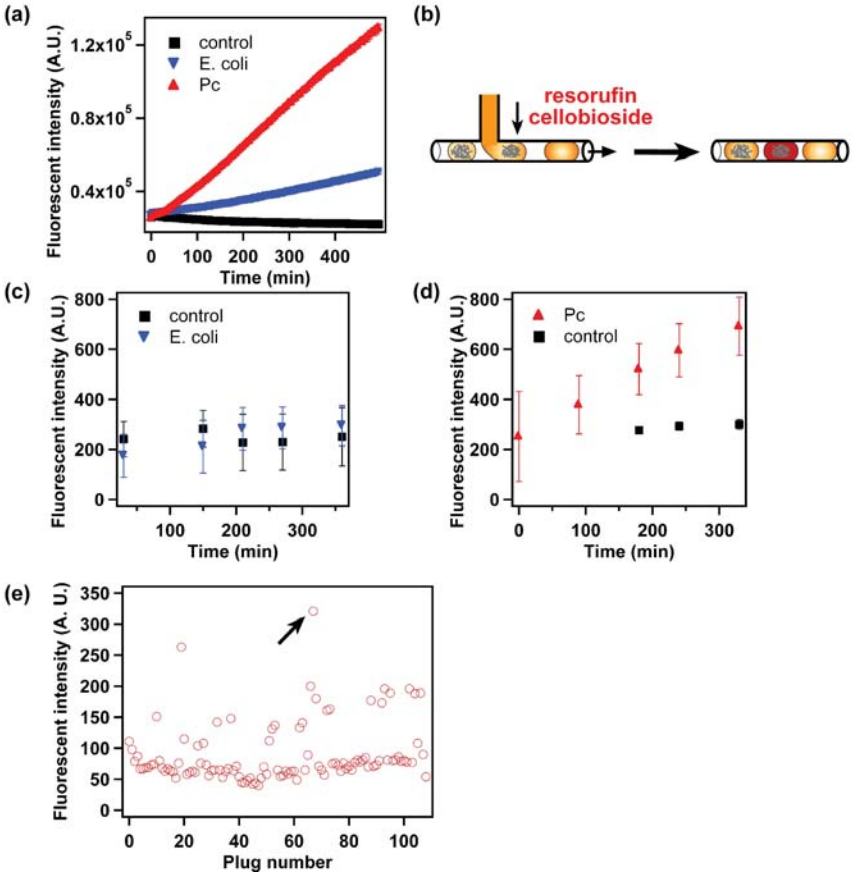


**Fig. S3.** Cellulase assay of bacteria was performed in plugs. (a) Kinetic profiles of fluorescence generation of *P. curdlanolyticus* and *E. coli* in a cellulase assay performed in a 96 well microplate measured by using a FLUOstar Omega microplate reader (BMG Labtech). (b) A schematic drawing of how the cellulase assay was performed in plugs. The fluorogenic substrate, resorufin cellobioside, was injected into each plug. Next the plugs were incubated to allow the cellulolytic reactions to occur, and then the red fluorescence of each plug was measured over time. (c) The kinetic profile of fluorescence generation of *E. coli* in a cellulase assay performed in plugs. The fluorescence of plugs containing *E. coli* increased gradually, but was still not significantly distinguishable from fluorescence in control plugs without any cells after 6 h incubation ($p=0.5683$). Error bars denote standard deviations (n=3, control; n=4, *E. coli*). (d) The kinetic profile of fluorescence generation of *P. curdlanolyticus* in a cellulase assay performed in plugs. The fluorescence of plugs containing *P. curdlanolyticus* increased rapidly, and was significantly distinguishable from fluorescence in control plugs without any cells after 5.5 h incubation ($p=0.0001$). Error bars denote standard deviations (n=3, control; n=16, *P. curdlanolyticus*). (e) Fluorescence intensities of the plugs in the array for Fig. 4a; the cellulase assay was performed on this array. The black arrow labels the plug containing *P. curdlanolyticus*, whose image was presented in Fig.

4a. Data presented in (c-e) are of the maximum intensity over the plugs minus 200 a.u. of background scattering.

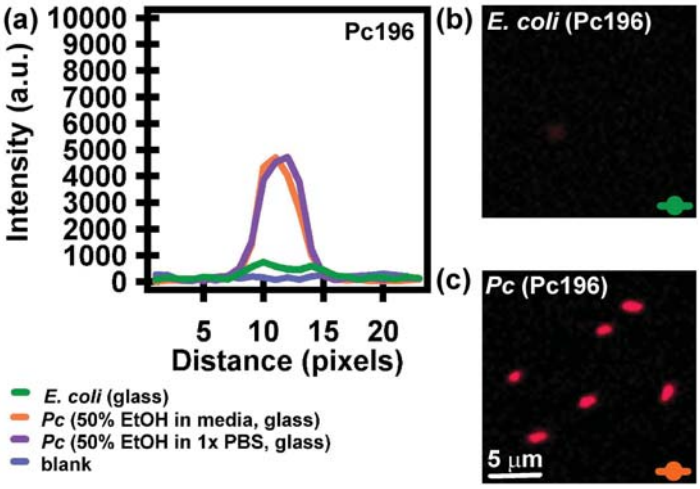


**Fig. S4.** Bulk cultures of *P. curdlanolyticus* and *E. coli* were stained by Fluorescence *In Situ* Hybridization using the probe Pc196 to identify *P. curdlanolyticus*. **(a)** Fluorescence intensity (a.u.) of the *P. curdlanolyticus*, *E. coli* and blank stained on a glass slide under different fixation conditions. **(b)** *E. coli* showed only background fluorescence when stained using the Pc196 probe. **(c)** *P. curdlanolyticus* was readily identified when stained using the Pc196 probe.

**Supplementary References**

1. L. S. Roach, H. Song and R. F. Ismagilov, *Anal. Chem.*, 2005, **77**, 785-796.
2. D. C. Duffy, J. C. McDonald, O. J. A. Schueller and G. M. Whitesides, *Anal. Chem.*, 1998, **70**, 4974-4984.
3. J. Q. Boedicker, L. Li, T. R. Kline and R. F. Ismagilov, *Lab Chip*, 2008, **8**, 1265-1272.