Supporting Information

Bacterial microcolonies in gel beads for high-throughput screening of libraries in synthetic biology

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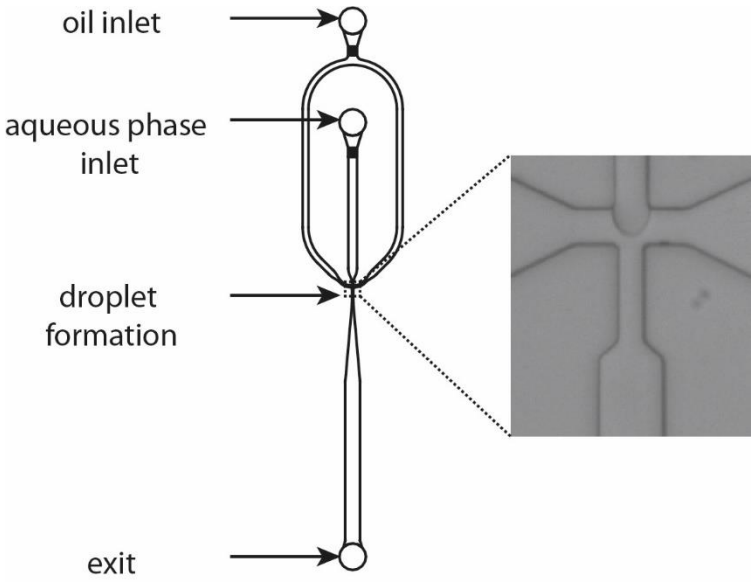

Figure S1. Design of the microfluidic device used for droplet generation. The device contains an inlet for the oil phase, an inlet for the aqueous phase (bacteria, agarose, medium) and an exit outlet. The droplets are formed at the flow-focusing geometry (picture inset). We used two sizes of the device: to produce droplets with a diameter of approximately 20 µm the channel width at the flow focusing part was 16 µm and the height of the channels was 20 µm. To produce droplets with a diameter of approximately 50 µm the channel width at the flow focusing part was 40 µm and the height of channels was 50 µm.

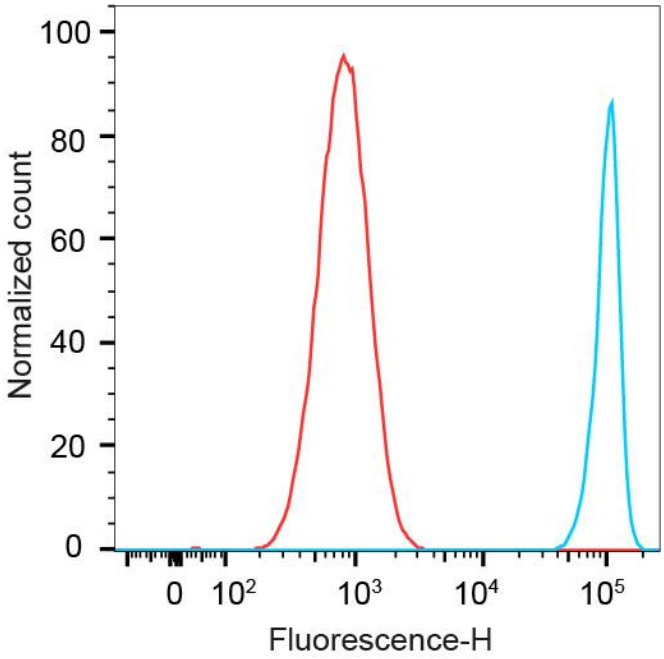

Figure S2. Variation in fluorescence of free cells and of microcolonies in beads. Red: *E. coli* cells (TOP10) constitutively expressing GFP (plasmid: pET-J23100-TetO-GFP-LVA) grown in solution (LB including ampicillin) for 8 h and measured by flow cytometry. Coefficient of variation (CV) = standard deviation / mean = 0.44. Blue: The cells were encapsulated into beads with a diameter of 50 µm, incubated for 8 h and measured by flow cytometry. The fluorescence of beads containing a microcolony is shown. CV = 0.21. This indicates that we did not introduce additional variation due to cell growth in the beads.

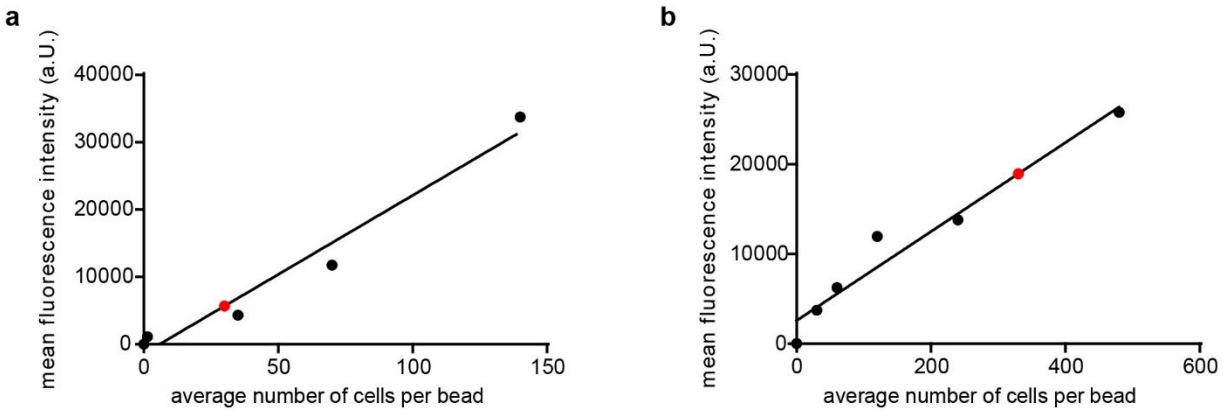

Figure S3. Estimation of number of cells per bead. We encapsulated *E. coli* cells constitutively expressing GFP (plasmid: pET-J23100-TetO-GFP-LVA) into beads at different cell densities. 0.4% sodium alginate was included as density matching agent to prevent settling of the cells in the syringe. We analyzed the beads by flow cytometry and plotted the mean of the fluorescence against the average number of cells bead encapsulated. We then performed a linear regression analysis. The red data points represent the fluorescence of beads that initially contained a single cell and were incubated for 4 hours. a) Beads with 20 µm diameter. From this analysis, we

estimate the number of cells in the incubated beads to be 30 cells. b) Beads with 50 µm diameter. From this analysis, we estimate the number of cells in the incubated beads to be 330 cells.

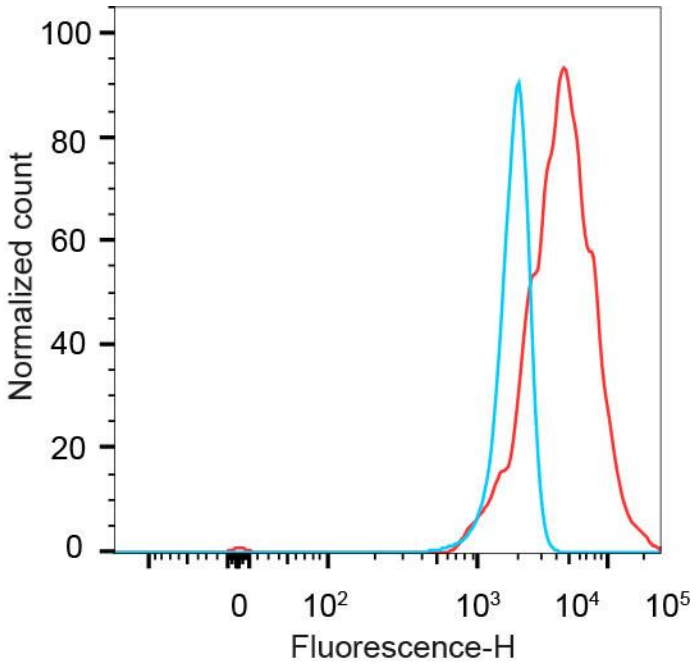

Figure S4. Incubation of beads in emulsion versus incubation in medium. We encapsulated *E. coli* TOP10 cells constitutively expressing GFP (plasmid: pET-J23100-TetO-GFP-LVA) into 20 µm beads. After the initial cooling of the emulsion on ice, we broke half of the emulsion sample into 500 µl LB medium (supplemented with 100 µg/ml ampicillin). The beads in the emulsion and in the LB medium were incubated for 5 h at 37 °C. Next, we cooled the beads again on ice. To remove free cells, we added the beads in LB onto a 10 µm filter (CellTrics, Partec) and the beads that did not pass the filter were resuspended in PBS. We also recovered the beads from the emulsion in PBS. We analyzed both samples by flow cytometry (FACSCanto) and gated for beads containing microcolonies. The fluorescence histograms of the two samples are shown. Blue: beads incubated in the emulsion, red: beads incubated in LB medium. The mean fluorescence of the blue histogram is 19’440 and that of the red is 49’549, indicating that more cells grew inside the bead when the emulsion was removed prior to incubation.

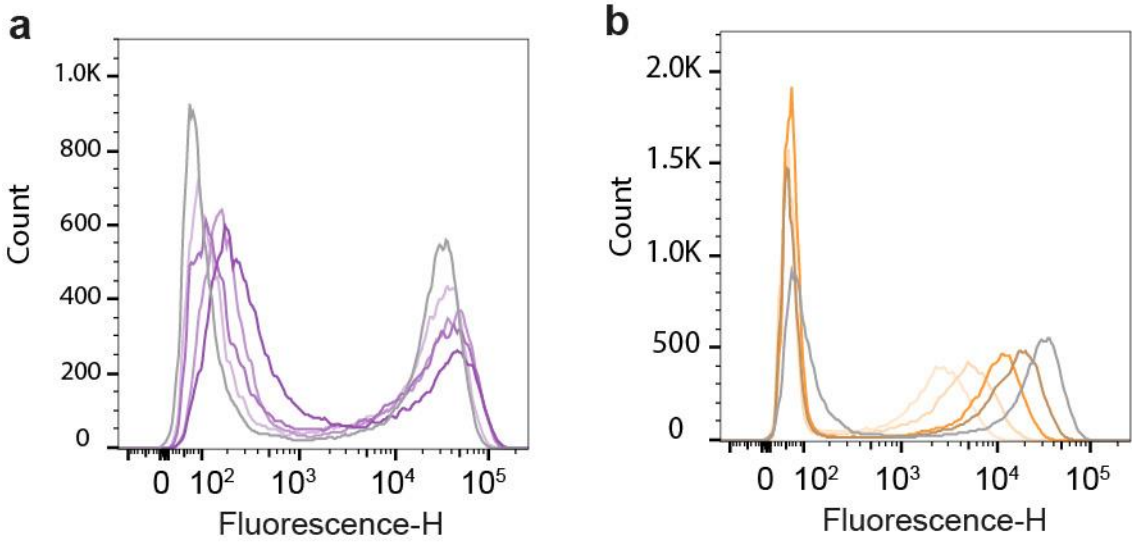

Figure S5. Flow cytometry analysis of individual pBAD promoter mutants. Mutants (TOP10 cells) displayed in Fig. 5 were grown in solution with 0.0005% (w/v) arabinose and individual cells were measured by flow cytometry. Grey: WT pBAD promoter. Purple and orange: Mutants. The darker the color, the higher the average fluorescence of the populations. a) Mutants from the ON "high" + OFF sorts (see Fig. 4) b) Cells from the ON "low" + OFF sorts (see Fig. 4). Mutations in the pBAD promoter can change the ratio of the ON and OFF subpopulations, as well as the positions of the ON and OFF peaks.

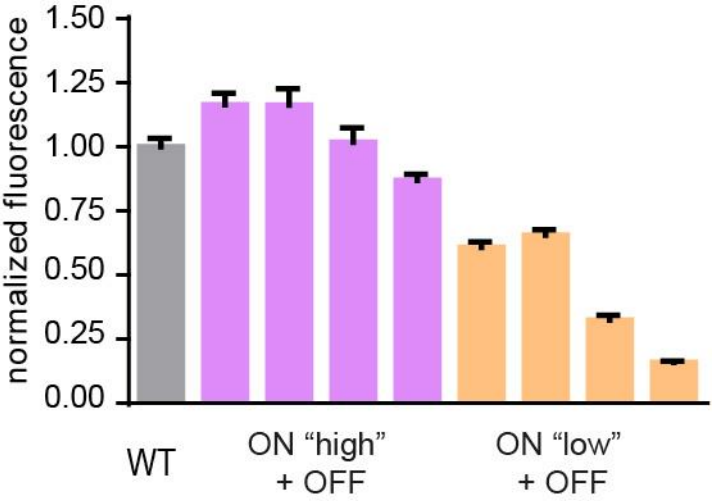

Figure S6. pBAD promoter mutants in a strain with homogeneous expression of the pBAD promoter. The plasmids from the mutants displayed in Fig. 5 were isolated and transformed into an *E. coli* strain where the native araE promoter is replaced by a constitutive promoter (Kogenaru, M., and Tans, S. J. (2014); *J Biol Eng 8*, 2). This results in a homogeneous cell population expressing genes under the control of the pBAD promoter, with a graded response to arabinose. The experiment of Fig. 5a was repeated with this strain. The relative fluorescence of the mutant promoters compared to the WT promoter is very similar as in the TOP10 strain (Fig. 5).