

Two distinct states of *Escherichia coli* cells that overexpress the recombinant heterogeneous β -galactosidase

Supplemental Figures and Tables

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Supplemental Table S1. The average ratio of the activities of the high-activity enzyme-containing cells to that of the low-activity enzyme-containing cells from 4 to 10 hrs after IPTG induction.

time ^a	Average ratio ^b
4 h	13.7±5.8
6 h	17.5±4.0
8 h	13.1±2.9
10 h	13.7±6.3

^aIPTG (0.5 mM final concentration) was added to cell culture at 0.6 O.D., and incubated for designated times.

^bThe ratios are the averages of three repeated experimental values.

Supplemental Table S2. Characteristics of proteins used in the IPTG removal study.

Protein	Description	Temperature (°C)	Incubation time (hour)	IPTG concentration (mM)
tBgl1	a kind of thermophilic glucosidase	28	20	0.5
tBgl3	a kind of thermophilic glucosidase	28	20	0.5
hCaf1	human poly(A) specific ribonuclease	10	36	0.05
hPop2	human poly(A) specific ribonuclease	10	36	0.05

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. S1. Scattering signals from Cell free PBS buffer (left) and Non-induced *E. coli* cells (right) analyzed by flow cytometry.

The majority of the background signals from PBS appeared in the lower left and only 0.43% of the background signals overlapped with the cell signals.

Supplemental Fig. S2. The *in vivo* activities of β -galactosidase.

The cells containing non-fusion form of the β -galactosidase were analyzed by flow cytometry. **0 h**: uninduced cells; **8 h**: induced with IPTG for 8 hours; **4 h + 4 h**: induced for 4 hr and then cultured in IPTG-free medium for another 4 hours.

Supplemental Fig. S3. Comparison of the effects of conditioned medium and fresh medium on the percentage of high activity enzyme-containing cells.

(A) β -galactosidase activity detected by flow cytometry.

(B) The percentages of high β -galactosidase activity population.

Supplemental Fig. S4. The effect of the O.D. value before induction on the percentages of two β -galactosidase activity cell populations.

The high and low activity cells in the R1 region are defined in Fig. 1A. (A) The β -galactosidase activity distribution identified by FL1-H (green fluorescence) using flow cytometry. The control cells are uninduced cells. (B) The percentages of two β -galactosidase activity populations. (C) the total β -galactosidase activities of the R1 region cells. Cells were cultured for 5.5 hr.

Supplemental Fig. S5. Effects of IPTG removal on the solubility of the heterogeneous proteins in *E. coli*.

Each of the heterogeneous proteins (A. hCaf1; B. hPop2; C. tBgl1 and D. tBgl3) was expressed using *pET28a* vectors and *BL21(DE3)* host strain and induced by IPTG for a given time at a given temperature, then IPTG was removed by washing the cells as described in *manuscript* and the cells were cultured for a designated time in IPTG-free LB medium. The incubation time lengths before and after IPTG removal were the same for each protein. The incubation times before and after IPTG removal and temperatures were given in **Supplemental Table S2**. The supernatant and precipitate fractions were subjected to SDS-PAGE. **UP**: precipitate fractions of unwashed-cells; **US**: supernatant fractions of unwashed-cells; **WP**: precipitate fractions of washed-cells; **WS**: supernatant fractions of washed-cells.

Figure S1

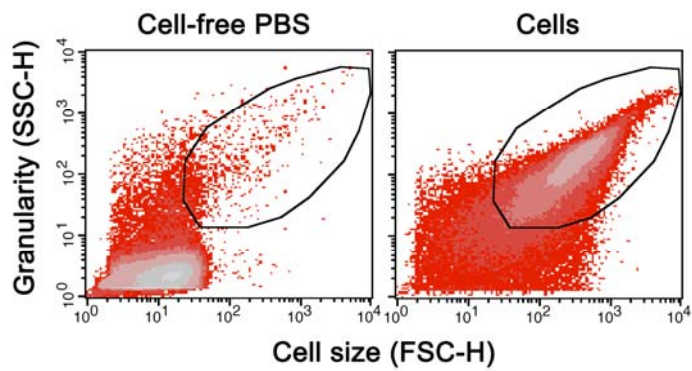


Figure S2

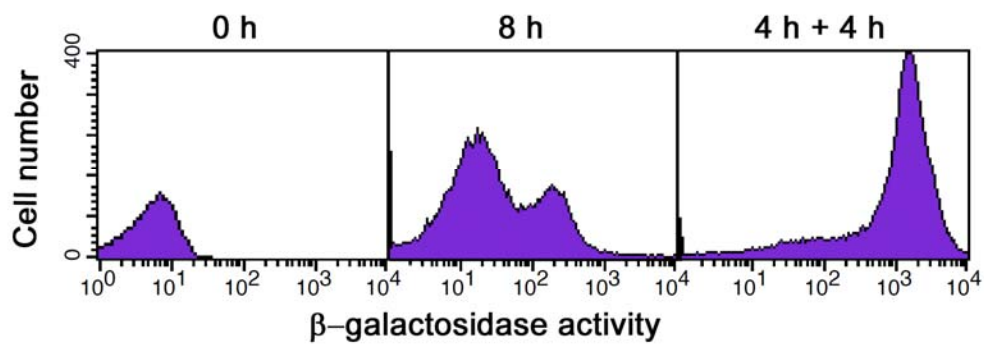


Figure S3

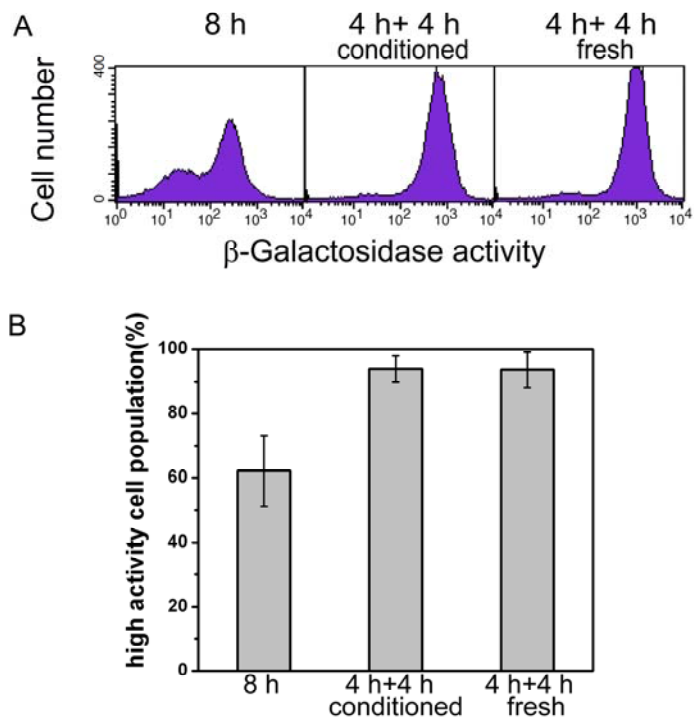


Figure S4

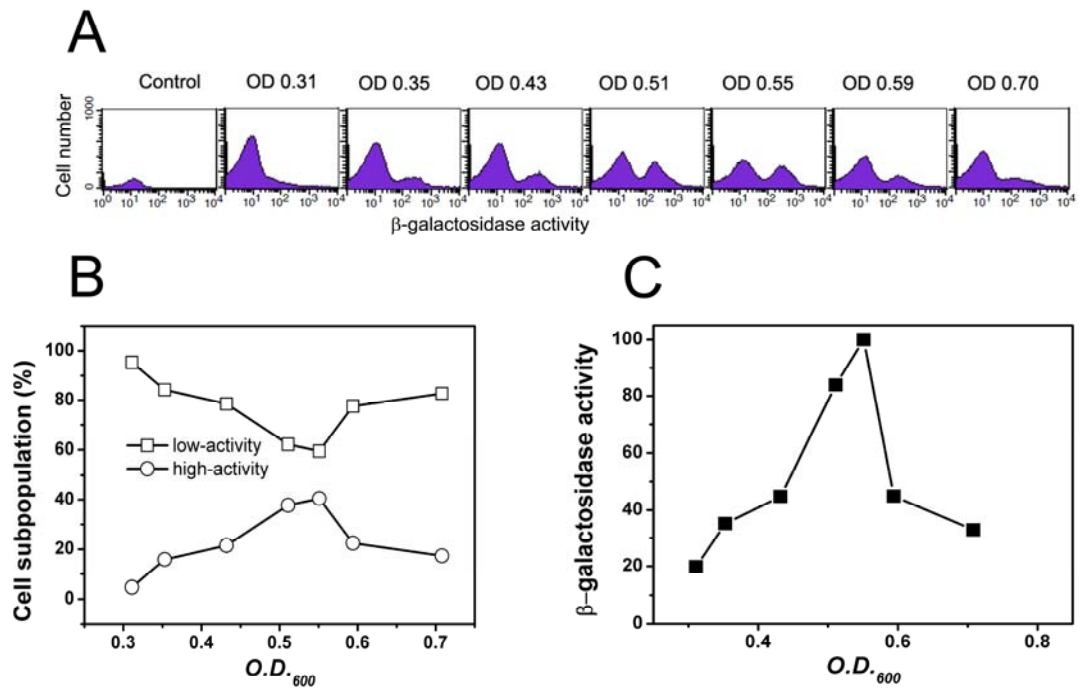


Figure S5

