[Supplementary material]

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Development of a high-copy-number plasmid via adaptive laboratory evolution of *Corynebacterium glutamicum*

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Table S1. List of point mutation on *parB* region

Clone number	Nucleotide Number	Original	Mutant	Codon exchange	Tandem repeat
8	34..49				GGCCTGATTCCTCCCC
10	34..49				GGCCTGATTCCTCCCC
6	175	G	T	GAA→TAA	
3	4	G	T	GAG→TAG	
4	61	C	T	CGA→TAG	
1	21	C	A	TGC→TGA	
2	25	C	A	TGC→TGA	
5	84	C	A	TGC→TGA	
7	84	C	A	TGC→TGA	
9	84	C	A	TGC→TGA	

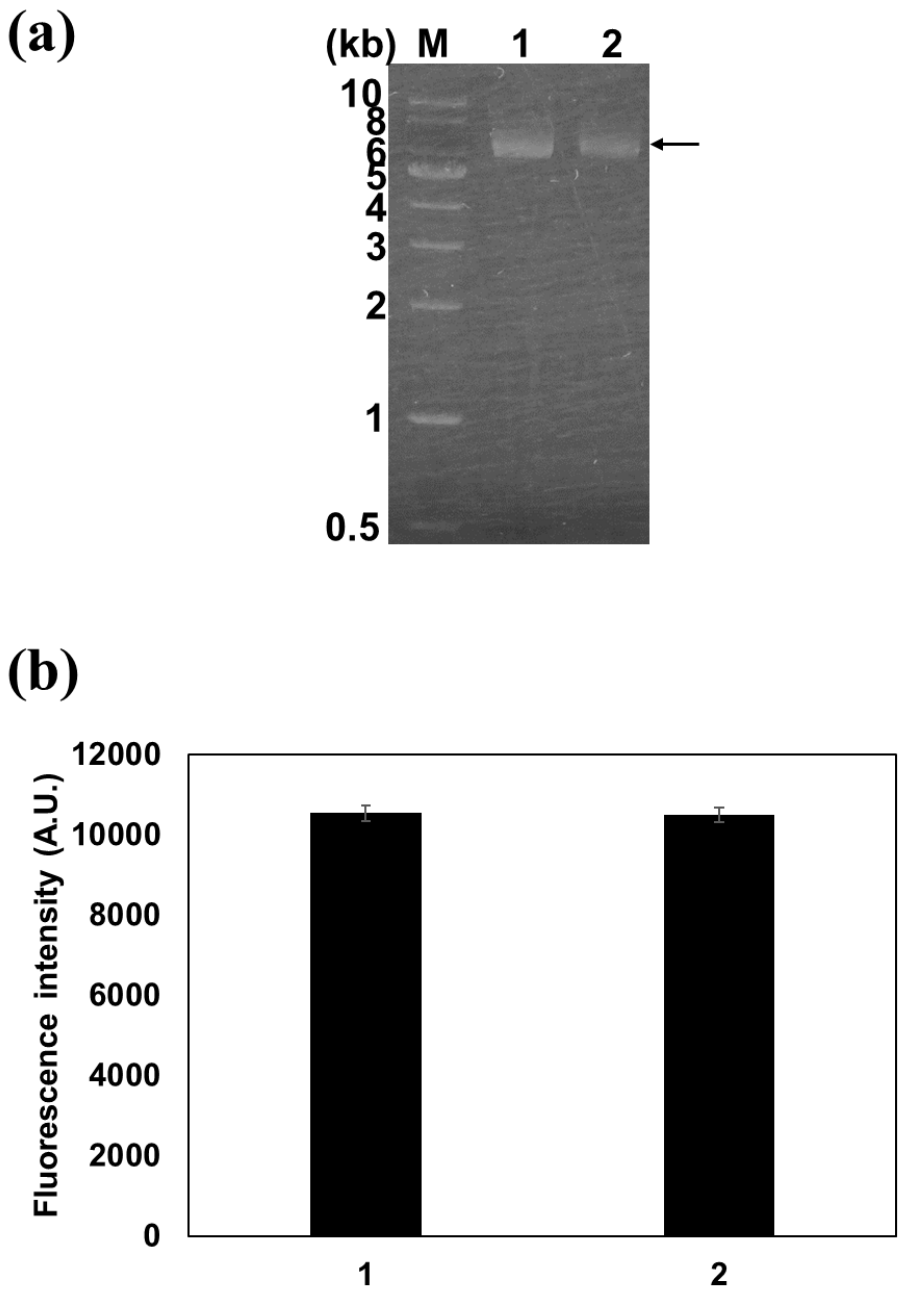

Fig. S1 Confirmation of difference between isolated clone and re-transformant clone. (a) Agarose gel analysis of plasmid prepared from the isolated clone (lane 1) and re-transformant clone with the isolated plasmid (lane 2). Lanes M represent DNA size markers (kb). Arrow indicates the band of plasmid (b) Fluorescence intensity analysis of the isolated clone (bar 1) and re-transformant clone with the isolated plasmid (bar 2).

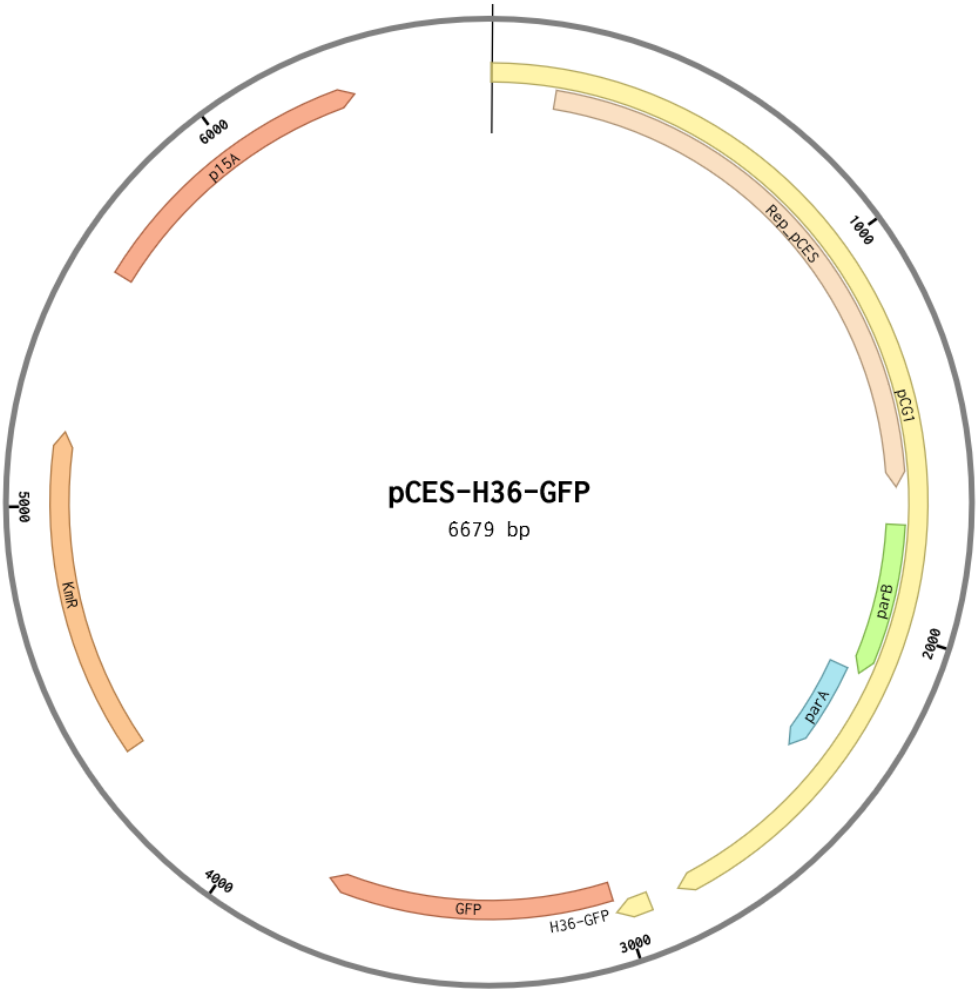

Fig. S2 Plasmid map of pCES-H36-GFP. pCG1 is cryptic plasmid from *C. glutamicum*. p15 is origin of replication of *E. coli*.

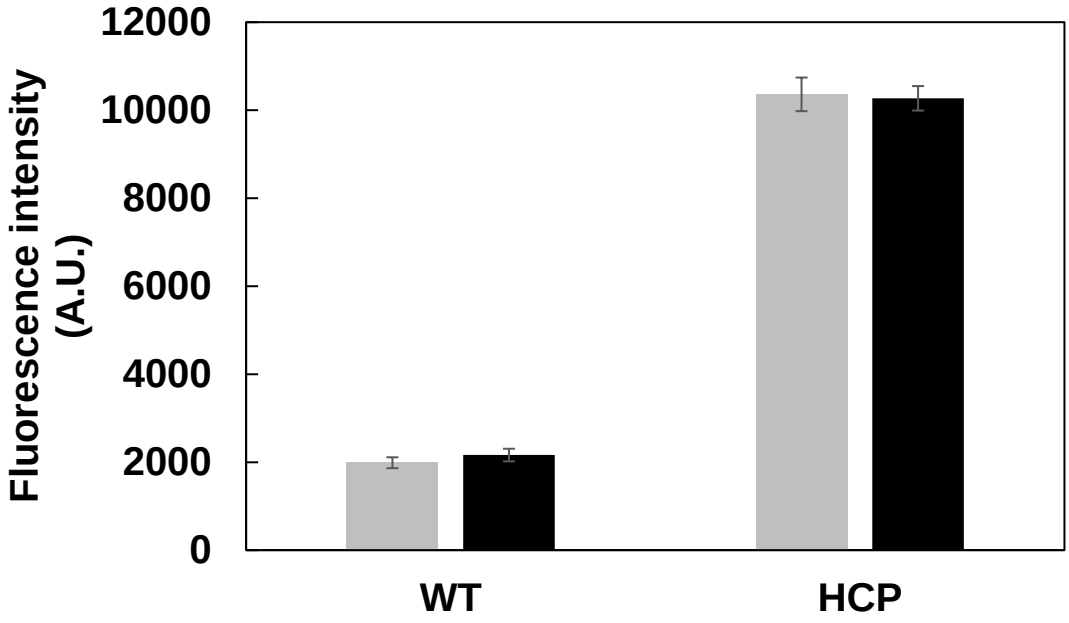

Fig. S3 Confirmation of plasmid segregational stability with or without antibiotics. Gray and black mean fluorescence intensity of cells with or without antibiotic during 60 generation cultivation. WT and HCP mean pCES-H36-GFP and pHCP-H36-GFP.

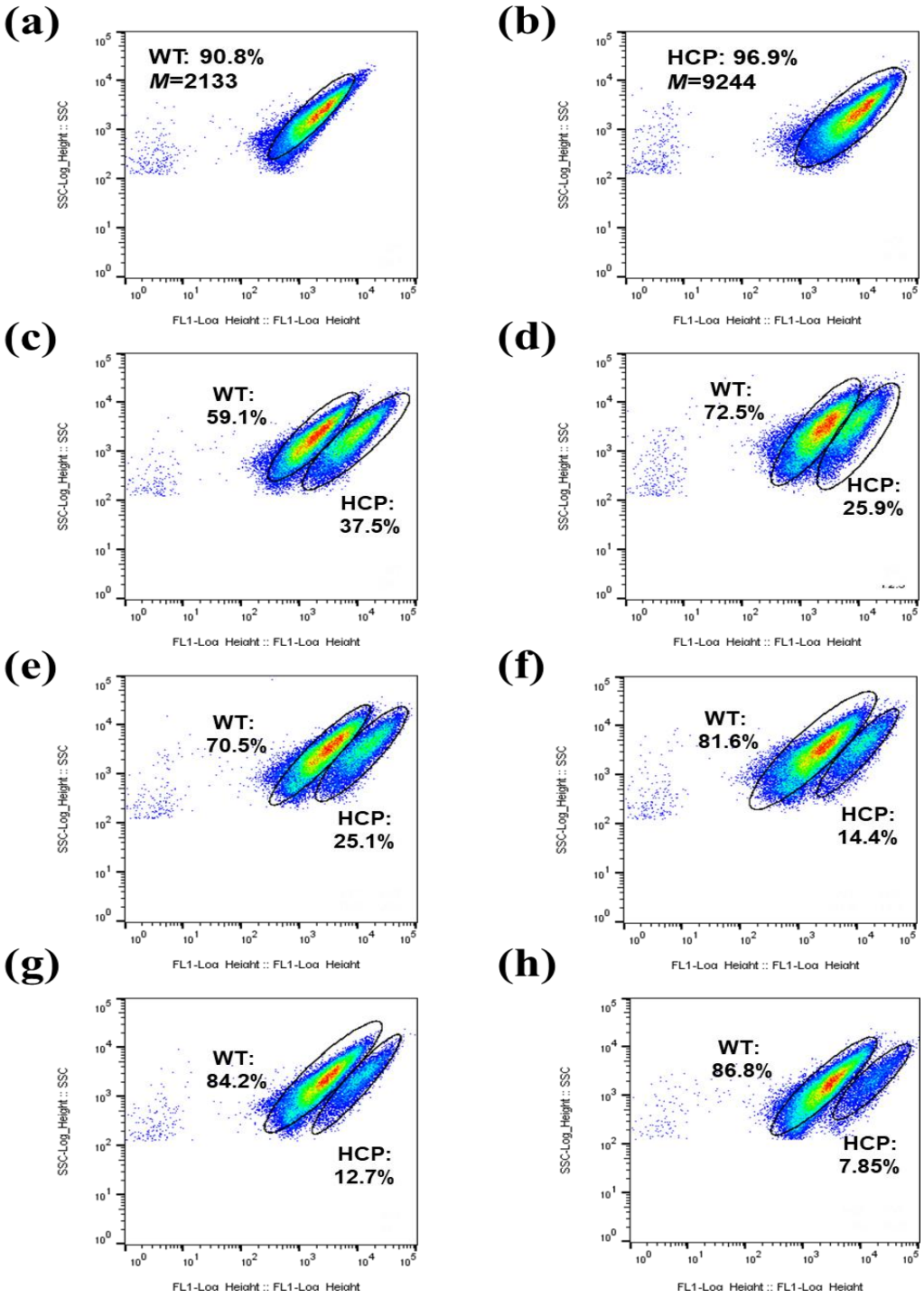

Fig. S4. FACS analysis of population changes during co-cultivation with cells harboring pCES-H36-GFP or CP-H36-GFP. Dot plots displaying the fluorescence intensity of GFP (Log Height) against the SSC-Log Height (cell size). (a) and (b) show result of cells harboring pCES-H36-GFP (WT) and pHCP-H36-GFP (HCP), respectively. (c) to (h) represent the FACS analysis of 1st round to 6th round. The percentage (%) in each histogram indicate the

percentage of WT or HCP population in total populations. *C. glutamicum* harboring pCES-H36-GFP or HCP-H36-GFP were inoculated into separate BHI medium. After 24 h, the cells were harvested at an OD_{600} of 4.0 and resuspended in 1 mL of brain heart infusion (BHI). Then, both cells were mixed with 1:1 ratio and were inoculated into fresh 50 mL of BHI in 250 mL flask. In every 12 h, 1mL of cells was transferred into fresh BHI and fluorescence intensity was analyzed by FACS.