Stable and tunable maintenance of multi-copy plasmid by antibiotics-free auxotrophic control

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Although benefits of multi-copy expression of the genes, plasmids require addition of antibiotics for their stable maintenance despite of the economic and environmental problems. Here, we developed the antibiotic-free auxotrophic plasmid maintenance system for stable and tunable gene expression by precise control of plasmid copy number (PCN) in *Escherichia coli*. To achieve this, we devised a system that expresses one of essential genes, *infA* encoding translation initiation factor (IF-1), on plasmid instead of chromosome. With this system, the gene expression was stably maintained for 40 generations (240 cells) with minimized cell-to-cell variation. Moreover, the PCN was rationally tunable in 5.6-fold by varying expression of *infA*. This antibiotic-free PCN control system was applied for engineering *E. coli* to produce itaconic acid and lycopene. Notably, antibiotic-free control of the PCN enabled significant improvement on the production of both itaconic acid (1.7-fold) and lycopene (2.0-fold) compared to that of the conventional system based on antibiotics. Collectively, the developed strategy could be a platform for production of value-added products in antibiotic-free cultivation.