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# Designing Genetic Circuits for Laboratory-Adaptive Evolution of Naringenin Production in E. coli

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## PURPOSE OF THE ABSTRACT

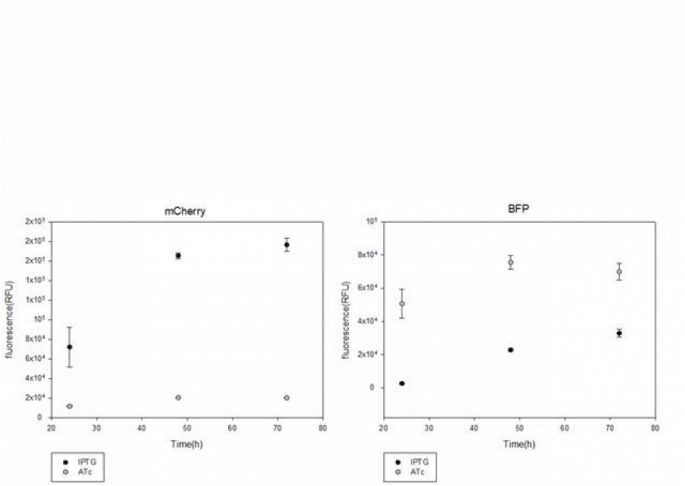
Microorganisms have been utilized in various ways to produce desired metabolites through metabolic engineering. However, artificially regulating pathways to increase production can be impractical when the pathways are complicated or not well-known. In such cases, laboratory evolution can be employed to increase the production of desired metabolites.

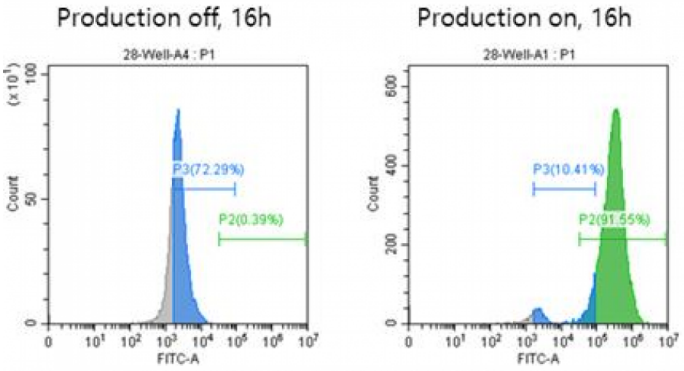
Laboratory evolution involves generating random mutations in the entire genome of microorganisms to obtain strains with new traits, then selecting those that produce a large amount of the target metabolite. This method is very useful in metabolic engineering as it can yield strains that produce large quantities of target metabolites even if the metabolic pathway is complicated or not well-known. However, a method for rapidly generating mutations causing evolution and easily selecting strains of large production is necessary.

To address this, we designed genetic circuits for mutation acceleration and strain selection. Various types of mutator devices were tested, each with different characteristics depending on the gene it contains. Each device showed different mutation rates and different levels of leaky expression. Several selection devices were also created, each responsible for different concentrations of target molecules. The naringenin sensor was used as an example. Transcription factor fdeR was utilized to recognize naringenin and activate the fdeA promoter. A UTR region was engineered for wide-range recognition. Antibiotic resistance and fluorescence protein genes were expressed only when the strain produced naringenin. A genetic toggle switch was used to control the operation of mutagenesis and selection. The toggle switch consisted of two cross-regulating transcription factors, lacI, and tetR. We integrated the toggle switch in the E.coli genome, and the switch maintain the state for 72 hours. By combining each device, it was possible to control the mutation state and the selection state repeatedly.

In conclusion, genetic circuits were devised for laboratory-adaptive evolution of naringenin. The genetic circuit consisted of three parts, each composed of a mutagenesis device, a selection device, and a toggle switch to control them. Mutagenesis devices were made of two types, considering mutagenesis ability and stability. The selection device consisted of an antibiotic resistance gene and a fluorescence gene based on a transcription factor, and it worked well if naringenin was present. The toggle switch regulated the two states by adding appropriate inducers. The combined genetic circuit can be effectively used during adaptive evolution to produce metabolites on a large scale using E.coli.

## FIGURES




### FIGURE 1

Toggle switch test

Integrated toggle switch circuit was stable over 72hour. IPTG and ATc was used to change each state.

### FIGURE 2

Selector device test

Selector device showed fluorescence only when the cell produced naringenin


## KEYWORDS

synthetic biology | genetic circuit | laboratory evolution | metabolic engineering


## BIBLIOGRAPHY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