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A combinatorial library of enzymes for anthocyanin biosynthesis toward designing of an artificial anthocyanin operon

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Many of biosynthetic pathways for useful chemicals of plant have been tried to transplant in bacterial cell for the purpose of increase production speed and quantity. Construction of a heterologous metabolic pathway in a bacterial cell is, however, still difficult as the number of required enzyme is getting bigger. To investigate a method for construction of metabolic pathway, we render a biosynthetic pathway of plant specific pigment, anthocyanin as a model. In our former work, a series of 9 enzymes required for production of peralgonidin, an anthocyanin compounds, in *Escherichia coli* were assembled in a plasmid as polycistronic operon form. However, any anthocyanin was detected in an *E. coli* harboring the operon, although production of naringenin, an intermediate metabolite, was observed. This implies that design for the artificial operon is necessary to produce peralgonidin. We then focus on relevant four enzymes required for peralgonidin synthesis from naringenin. Since, in an artificial operon profile of expression level of the genes is expected as monotonical decrease from promoter [1, 2], we thus tried *in vitro* production of peralgonidin with different amount of the enzymes mimicking possible 24 (=4!) operons to find out optimal 4-enzyme ratio for production. As a result, specific combinations showed significant production of peralgonidin. Now we are making several artificial operons including four enzyme genes with optimal gene orders according to the enzyme combinations by Ordered Gene Assembly in *Bacillus subtilis* (OGAB) method [3].

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