# Reconstruction of Coenzyme $B_{12}$ Production Pathway in an Engineered *Escherichia coli*

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Coenzyme $B_{12}$ is one of the largest and the most structurally complicated chemical in nature. Because it plays essential role in various metabolic functions and in many enzymatic reactions as a cofactor, its economic production are highly meaningful. Due to its complex structure, chemical synthesis is virtually impossible. Instead, $B_{12}$ can be obtained from native producers such as *Pseudomonas denitrificans* and *Klebsiella pneumoniae*. However, a lack of genetic engineering tool for these microorganisms limits us to further develop $B_{12}$ high producer. Rather than engineering native producers, reconstruction of $B_{12}$ pathway in genetically well-known microorganisms such as an *Escherichia coli* could be more conceivable to achieve high production. In this study, we applied multiple synthetic biology based approaches in *E. coli*. Initially, the genes for $B_{12}$ biosynthesis in *P. denitrificans* were introduced; the genes were expressed under a strong promoter and optimized 5' untranslated regions (5'-UTR) for maximum expression. Then, an amplification of precursor pool and developing an efficient screening device allowed further strain improvement for increased $B_{12}$ production.