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E. coli Host Engineering for Efficient Enzyme Discovery

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The bacterial ribosome consists of three rRNA molecules and 57 proteins and plays a crucial role in translating mRNA-encoded information into proteins. Because of the ribosome's structural and mechanistic complexity, it is believed that each ribosomal component coevolves to maintain its function. Unlike 5S rRNA, 16S and 23S rRNAs appear to lack mutational robustness, because they form the structural core of the ribosome. However, using *E. coli* Δ7 (null mutant of operons) as a host, we have recently shown that an active hybrid ribosome whose 16S rRNA has been specifically substituted with that from non–*E. coli* bacteria can be reconstituted *in vivo*.

To investigate the mutational robustness of 16S rRNA and the structural basis for its functionality, we used a metagenomic approach to screen for 16S rRNA genes that complement the growth of *E. coli* Δ7. Various functional 16S rRNA genes were obtained from the Gammaproteobacteria and Betaproteobacteria lineages (with minimal identity of ~80%) [1].

Based on this discovery, we investigated how ribosomal mutation affects gene expression. To this end, we used a series of reporter genes that are poorly expressed in native *E. coli* host. They were then introduced into mutant *E. coli* library that carry metagenomically retrieved 16S rRNA genes, and the expression levels were evaluated. As a result, some mutants expressed gene more efficiently than the native *E. coli* host. Thus ribosome engineering may be a feasible approach to solving the expression problem in metagenomic library screening, and should accelerate the enzyme discovery process [2,3].

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