Expression Attenuation for Optimal Functional Expression of eGFP in *E.coli* by Modification of N-terminal Sequence

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Although green fluorescent protein (GFP) and its functional variants including enhanced GFP (EGFP) are widely used as a reporter gene in the various organisms, significant fraction of the insoluble form is still expressed especially in E. coli. This is a major hurdle for quantitatively reporting the proper expression level. Previously, several studies revealed that N-terminal sequences downstream of the start codon affected the expression level and there is a strong correlation between the expression level and the formation of the protein aggregates. In this study, we designed the N-terminal sequence variants of EGFP for the quantitative reporting system in E. coli. First, the minimal functional region of the EGFP was found to be starting at amino acid 5 in E. coli. Additionally, from the single amino acid deletion mutants, the deletions at position 3 and 4 were found to reduce the insoluble fraction formation since the secondary structure of mRNA around Shine-Dalgarno (SD) sequence as well as the start codon suppressed the translation level. From the expression under the various T7 promoter mutants, the EGFP variant obtained in this study linearly reported the expression level by its fluorescence.