Invention of a Device for Mutational Analysis of Bacterial Gene Expression

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Gene expression is the process by which the information of genes is used to direct the activities of cells in all living things. It involves the transcription of DNA into mRNA and the translation of mRNA into protein. Translation initiation is controlled by ribosome binding sites found in mRNA molecules that allow them to base pair with RNA in the ribosome. The current project was to invent, construct, and test a new molecular cloning system called rClone that uses Golden Gate Assembly to enable mutational analysis of ribosome binding sites. The capability of rClone to facilitate research was demonstrated by using it to prepare libraries of mutated ribosome binding sites. Mutant libraries based on a synthetic bicistronic ribosome binding site were also built. Exploration of the libraries involved random selection of 127 mutants, determination of their DNA sequences, and measurement of their effects on the expression of a RFP reporter gene. The results yielded ribosome binding site consensus sequences that were compared to a consensus sequence for naturally occurring E. coli ribosome binding sites. The consensus sequences were tested with an RFP reporter gene and a blue chromoprotein reporter gene. The results showed higher ribosome binding site strengths for the blue chromoprotein reporter gene. Examination of the sequence of the RFP reporter gene led to a new hypothesis for an anti-ribosome binding site in the RFP gene. Overall, the project results illustrate the power of rClone for mutational analysis of bacterial gene expression.