Mutational Analysis of Bacterial Gene Regulation

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Transcriptional promoters play an important role in bacterial gene regulation. Many promoters have a conserved DNA sequences called the -35 element and the -10 element. This study was to introduce mutations into the -35 element of a high efficiency promoter called Ptac and measure their effects on gene expression. The mutated promoters were studied using a new system for promoter research, pClone. The mutated promoters were cloned upstream of a red fluorescence protein (RFP) reporter gene and transformed into E. coli. A total of 81 clones with mutated -35 regions was collected. The DNA sequence of the 81 clones was determined. The transcriptional efficiency of each of the clones was determined by fluorometric measurement of red fluorescence. The efficiency of the clones covered a wide range. A consensus sequence was determined for the -35 element of the 81 clones. The new consensus sequence is different from that published in research papers and textbooks. The consensus sequence was tested by cloning four sequence variants derived from the consensus into Ptac. The results showed that the consensus functioned with an efficiency similar to that of the published consensus. The four variants also functioned in two other promoters with different sequence contexts. The results of the study were contributed to three databases for use by the synthetic biology community.

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