Regulation of Rolling Circle Amplification

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When M13 phage enters in E.coli, it replicates itself using E.coli's enzymes such as DNA polymerase, RNA polymerase, and ligase. This is called rolling circle replication. There are three stages in rolling circle replication. When production of single strand DNA is plotted simply, longer stage 2 is favorable. In this rolling circle replication, p5 concentration is a branch point between stage 2 and stage 3. If the accumulation of p5 is fast, shift to stage 3 occurs fast. However, accumulation of p5 is slow, shift to stage 3 occurs lately. It means that we can get longer stage2. We engineered 5' UTR of protein V in M13 phage and screened 4 most efficient mutants. Production of M13 phage increased to 6.03-fold, and ssDNA production increased in 5.66-fold. Furthermore, we measured the engineered spacer expression levels by fusion of protein V with GFP. The fluorescence of the good producers is lower than the bad producers and the wild type. Also, the artificial expression of P5 using an inducible promoter shows that the concentration of P5 is inversely proportional to M13 phage and ssDNA production.