A Thesis for the Degree of Ph.D. in Engineering

# Compartmentalized replication for directed evolution of DNA polymerase and transcriptional regulator

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# Thesis Abstract

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Thesis Title

Compartmentalized replication for directed evolution of DNA polymerase and transcriptional regulator


Thesis Summary

Compartmentalized replication is a powerful method for directed evolution of proteins by using *in vitro* compartmentalization (water-in-oil emulsion) for genotype-phenotype linkage and PCR amplification for selection. This method was first developed for directed evolution of several DNA polymerases by compartmentalized self-replication (CSR), and then applied to two other proteins T7 RNA polymerase and tRNA-synthetase by compartmentalized partnered replication (CPR). However, the utility of CSR and CPR is somewhat limited by low efficiency of emulsion PCR with the wild-type *Taq* DNA polymerase so far used. To overcome this problem, in this study, I first performed engineering of *Tth* DNA polymerase with improved efficiency by using CSR. Next I applied this improved DNA polymerase for directed evolution of an arsenic-responsive transcriptional regulator by using CPR.

(1) *In vitro* selection of modified DNA polymerase I from *Thermus thermophilus* (*Tth* pol) using CSR.

Although PCR is an essential tool in the biological sciences, several limitations and problems remain, including long run times, low amounts of product, and false negative results due to inhibitors. In this study, I developed a *Tth* DNA polymerase mutant suited for CSR. First I modified the wild-type *Tth* pol by (i) deletion of the N-terminal 5' to 3' exonuclease domain, (ii) fusion with the DNA-binding protein Sso7d, (iii) introduction of four known effective point mutations from other DNA polymerase mutants, and (iv) codon optimization to reduce the GC content. Consequently, I obtained a mutant that provides higher product yields than the conventional *Taq* pol without decreased fidelity.

(2) Development of a novel dual selection system based on CPR and sacB for directed evolution of arsenic-responsive transcriptional regulator.

Arsenic is a well-known toxin that holds a constant threat to a large population worldwide. My ultimate goal is to develop an arsenite-inducible biosensor with the best possible sensitivity and specificity. In order to evolve the *ars* operon of *E. coli*, I intend to apply CPR, in which the *ars* operon is coupled with expression of *Tth* pol mutant described above. First I illustrated the use of genetic switch in designing the arsenic responsive whole cell biosensor in *E. coli*. Next, I developed a dual selection system based on CPR and negative selection using sacB for directed evolution of arsenic regulator (repressor) gene in this genetic switch.