Staggered extension PCR combines beneficial mutations without mutations

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Directed evolution stands on the advantages of DNA recombination to accumulate beneficial mutations while simultaneously removing deleterious mutations, which may greatly accelerate the evolution of a protein of interest toward a specific function. DNA shuffling, the first combinatorial recombination tool was enormously effective for this purpose but one drawback at least is the generation of unwanted mutations during the re-ligation PCR. The errors may ruin the optimal recombination of beneficial mutations. Conceptually similar but easier recombination protocol is the staggered extension PCR (StEP) developed by F. A. Arnold and coworkers. In this work we tried the StEP to shuffle selected mutations on *Symbiobacterium toebii* TPL variants. Taq and Vent among four different DNA polymerases were found to amplify and shuffle the genetic variations successfully. However with Taq polymerase, which is used generally in DNA shuffling, unexpected mutations were detected from two of ten sequences, while no mutation was found with Vent. In *Symbiobacterium toebii* TPL, the high accuracy StEP was very useful to combine muations for high activity and high stability originating from different variants. That is to say, selected hits from the StEP library were clearly improved in both the activity and thermostability, and resultantly comparable to a highly active *Citrobacter freundii* TPL.

[This project was supported by Cleaner Production Program 10007946 of NCPC]