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COMPLETE GENOME ANALYSIS AND TARGETED GENE DISRUPTION IN THE HYPERTHERMOPHILIC ARCHAEON, *THERMOCOCCUS KODAKARAENSIS*

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The genus *Thermococcus*, comprised of sulfur-reducing hyperthermophilic archaea, belongs to the order Thermococcales along with the closely related genus *Pyrococcus*. The members of *Thermococcus* are ubiquitously present in natural high temperature environments, and are therefore considered to play a major role in the ecology and metabolic activity of microbial consortia within hot-water ecosystems. To obtain insight into this important genus, we have recently determined and annotated the complete 2,088,737-base genome of *Thermococcus kodakaraensis* strain KOD1. A total of 2,306 coding DNA sequences (CDSs) have been identified, among which half (1,165 CDSs) are annotatable whereas the functions of 41% (936 CDSs) cannot be predicted from the primary structures. Comparative genomics clarified that 1,204 proteins, including those for information processing and basic metabolisms, are shared among *T. kodakaraensis* and the three *Pyrococcus* spp. On the other hand, among the set of 689 proteins unique to *T. kodakaraensis*, there are a number of intriguing proteins that might be responsible for the specific trait of the genus *Thermococcus*, such as proteins involved in additional pyruvate oxidation, nucleotide metabolism, and an improved stress response system.

We have also recently developed a gene disruption system for *T. kodakaraensis* by utilizing a *pyrF*-deficient mutant KU25 as a host strain and the *pyrF* gene as a selectable marker. To achieve multiple genetic manipulations for more advanced functional analyses of genes *in vivo*, we first constructed a new host strain KU216 (Δ*pyrF*) by specific and almost complete deletion of endogenous *pyrF* through homologous recombination. Secondly, a new host-marker combination of a *trpE* deletant KW128 (Δ*pyrF*, Δ*trpE*::*pyrF*) and *trpE* gene was developed. The system made it possible to isolate transformants through a more simple selection procedure as well as to deduce the transformation efficiency, overcoming practical disadvantages of the first system. Furthermore, repeated utilization of the counterselectable *pyrF* marker was established through its excision by pop-out recombination. Both endogenous and exogenous sequences could be applied as tandem repeats flanking the marker *pyrF* for pop-out recombination. A double deletion mutant KUW1 (Δ*pyrF*, Δ*trpE*), constructed with the pop-out strategy, was demonstrated to be a useful host for the dual markers, *pyrF* and *trpE*. The transformation systems developed here now provide the means for extensive genetic studies in hyperthermophilic archaea.