Development of transposon mutagenesis techniques for the genome-scale analysis of gene function in the hyperthermophilic crenarchaeon *Sulfolobus islandicus*.

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Introduction: Sulfolobus islandicus, an aerobic hyperthermoacidophilic crenarchaeon which grows optimally at 80°C and pH 2 in terrestrial solfataric springs around the world, has been developed as a novel model organism to study the unique biology of Crenarchaea. While traditional reverse genetic tools such as homologous recombinationbased knock-in and knock-out techniques have been developed recently to study the function of individual gene, highthroughput analysis of gene essentiality/non-essentiality on the genome scale has been hampered by the lack of sophisticted genetic approach. Here, we describle the development of a method for rapidly generating large numbers of transposon insertion mutants in S. islandicus. To vadidate this approach, we preliminarily analyzed a mini-library containing around 200 transposon insertion mutants by employing the high-throughput next generation sequencing (Tn-Seq) and it turns out that each mutant has a unique insertion of transposon. The application of the Tn-seq to analyze the libraries with around 20,000 transposon-inserted mutants (cover the 99.9% of the whole genome) will aid in revealing unexplored genetic determinants and the underlying mechanisms of various biological processes especially the DNA repair, replication and recombination, in the S. islandicus.