

**Strategies for Expression of Foreign Genes in *Thermus thermophilus* :
The Case Study of the Deinococcal Tryptophan Synthase Gene.**

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There are several reports of expression of foreign genes in *Thermus thermophilus*. Here I discuss several factors which we should take into account by reviewing the case of the deinococcal tryptophan synthase gene.

T. thermophilus is taxonomically close to the mesophilic radioresistant bacterium *Deinococcus radiodurans*. The G + C contents of the chromosome DNA of both bacterium are as high as 70%. *Deinococcus* would be a suitable mesophilic counterpart for *Thermus* to investigate the thermostability of proteins because the effect of high G + C contents to amino acid contents can be ignored.

The amino acid sequence of the tryptophan synthase alpha subunit of *T. thermophilus* shows highest homology (51.2 %) to *D. radiodurans* sequence among known genes. The *trpA* gene which codes for tryptophan synthase alpha subunit of *D. radiodurans* was amplified by PCR and was cloned onto an *E. coli* plasmid pUC18. The cloned gene was then introduced into a *trpA* deletion mutant strain of *T. thermophilus* HB27 with a *Thermus* plasmid vector pYK225 which contains a thermostable kanamycin resistance gene as a selection marker. The wild type *D. radiodurans* *trpA* gene complemented a *trpA* deletion mutation of *T. thermophilus* at 55C but not at 60C.

The *trpA* gene of *D. radiodurans* was amplified by mutagenic PCR and was introduced into a *trpA* mutant of *T. thermophilus* with pYK225 plasmid. Trp⁺ transformants were selected at 62C. The plasmid DNA pool was prepared from the mixed Trp⁺ transformants. The mutant *trpA* genes were amplified by PCR and were shuffled by the method of Stemmer. The shuffled DNA was introduced into a *trpA* mutant of *T. thermophilus* and Trp⁺ transformants were selected at 67C. Three additional cycles of shuffling and selection at restrictive temperature were done at 70C, 74C and 78C. The thermostable mutant *trpA* genes from Trp⁺ transformants at 78C showed 11 to 14 amino acid replacements.