Directed Evolution of Thermostability in Psychrophilic Luciferases; Design & Strategy

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Background: For many years researchers have attempted to understand structure-function relationships responsible for thermostability and definition of an enzyme's optimal and functional temperature range. While no general rules have emerged, research has focused on either comparisons of mesophilic proteins with their extant thermophilic counterparts – which have many amino acid differences, and between different protein fold families – which may vary in the structural strategy employed to achieve thermostability. To obviate the need to compare across families or through genetic drift, we develop a methodology to take psychrophilic enzymes of the same protein fold family (alpha beta barrels) and evolve thermostable characteristics.

Methods: Using classical molecular biology techniques we designed two shuttle vectors containing a bacterial luciferase from the psychrophile *vibrio harveyii* for expression and selection in both *e. coli* and *thermus thermophilus*, identified and optimized a selection range and transformation procedures for the high temperature expression strain.

Results: Growth rates for the high temperature expression strain were measured and optimal selection ranges were determined. Sequence of expression vectors was verified.

Discussion: The maximum temperature range observed for expression and selection was found to be 42 °C for *e. coli.* and 50 to 75 °C in *thermus thermophilus*. Optimal conditions for high throughput transformations are presented. Preliminary directed evolution results suggest that this is a viable system for selection of thermostable variants.

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